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Study on development of pitaya fruit (Hylocereus undatus) incorporated ice cream; an alternative solution to the pitaya cultivators in Sri Lanka

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Abstract - Study was conducted to determine the composition of pitaya (*Hylocereus* sp.) pulp, to develop an ice-cream incorporated with pitaya pulp and to evaluate the shelf life of the pitaya pulp incorporated ice cream. Ice-cream samples were prepared with three different proportions of pitaya pulp, 12%, 15% and 18% (w/w). A sensory evaluation was done by seven-point Hedonic scale on 20 semi-trained panellists. Physico-chemical and microbial analyses were conducted during storage. The ice-cream with 12% (w/w) pulp yielded the highest consumer preference.

Keywords - Pitaya, Vitamin C, Ice-cream, value addition

Introduction

Pitaya is the fruit of climbing wine cactus species of the genus *Hylocereus* sp. (sweet pitayas) commonly referred to as dragon fruit. It has received worldwide recognition not only as an ornamental plant but also as a fruit crop. Currently, it is cultivated in at least 22 countries in the tropics including Australia, Cambodia, China, Malaysia, Thailand, Bangladesh and Sri Lanka (Mizrahi and Nerd, 1999; Nerd et al., 2002; Nobel and Barerra, 2002). A Pitaya fruit fetches a higher price than a durian, the "king of fruits" in Southeast Asia. The agronomic practices are easier and less expensive, with fewer pests and diseases. The biggest advantage however is that it will grow for about 20 years, and one hectare could accommodate about 800 dragon fruit plants. It is a fast return perennial fruit crop with full production after five years (Gunesena et al, 2006).

Pitaya fruit is perhaps most common as the red pitaya fruit (red flesh or red pulp), sweet pitaya fruit (with creamy pulp) and a delicate aroma. To prepare for consumption, the fruit is cut open to expose the flesh which is eaten raw. It is mildly sweet and is known to be low in calories. The seeds are eaten together with the flesh, have a nutty taste and are rich in lipids (Abdul Azis Ariffin et al., 2008).

Pitaya fruit is known to be rich in nutritive value (Taiwan Food Industry Development and Research Authorities, 2005) with the pulp containing 82.5-83% moisture, 0.16-0.23% protein, 0.21- 0.61% fat, 0.7-0.9% fiber, 6.3-8.8 mg calcium, 30.2-36.1 mg phosphorous, 0.5-0.61 mg iron and 8-9 mg vitamin C.

The shelf life of pitaya fruit is drastically reduced with the movement of fruits from in and out of cold storage (Gunasena et al, 2006). Evidence show that after harvesting the respiratory rate decreases and the weight loss increases showing visible shriveling within eight days of storage (Arevalo-Galarza and Ortiuz-Herrnandes, 2004) leading to post harvest losses, therefore, alternative methods are required to reduce the post harvest losses to extended the shelf-life as of the fresh fruit.

The main objectives of this research were (i) To analyze the proximate composition of pitaya fruit pulp and pitaya incorporated ice cream. (ii) To assess the nutritional quality, sensory attributes and storage stability of the prepared ice cream. (iii) Reduce the post harvest economical losses of pitaya fruit and to

motivate the cultivators to produce more to reach shelf sufficient stage.

Methodology

Collection of Samples

Eighty fresh ripened pitaya fruits were purchased from Makandura farm located 1.5 km from department of Food Science and Technology and transported to Food Processing laboratory of Department of Food Science and Technology, Faculty of Livestock Fisheries and Nutrition, Wayamba University of Sri Lanka where it kept for a day under the refrigeration condition.

Chemical analysis of dragon fruit pulp

The Moisture of pitaya pulp was determined by drying at 100-105°C for 24 hours (AOAC 2004,). The acidity was expressed as anhydrous citric acid after titrating with 0.1M NaOH and the pH was measured by a pH meter (metrohm, mode 780, Dutchland) The ascorbic acid content in the product was estimated by a titrimetic method (Brody, 1994). An AOAC method (2004) was used to determine ash content of the sample. Total soluble solids (TSS) were determined by using a refrectometer (N-4E, Japan).

Processing of pitaya pulp incorporated ice cream

Pitaya pulp was separated from the skin by peeling or scooping out the fruity flesh. They were cut into (1x1cm) small pieces, and were blended for 2 minutes to obtain a homogenous mixture. All pulp thus collected was bundled into a muslin cloth and the content was immersed in hot water at 63°C for 2 minutes. Three different formulations of ice cream were made (Table 1) by mixing of fresh milk, whipping cream and sugar and then adding egg yolk and beating to obtain a homogenized liquid mixture meanwhile different proportion of fruit pulp was added to the three formulations. Ice cream mix was kept under refrigeration condition at 4°C for 3 hours for aging, stabilizer (cremodan) was added to the mix and beating was continued to incorporate air. Creaming was continued in an ice cream maker (CICM, mode 1700, Italy) for around one hour. The final product was dispensed into 300 small cups (150mL) and was kept under frozen condition (-14^{0}C).

Table 1:
Formulation of pitaya fruit incorporated ice cream.

Ingredients	T1	T2	T3
Fresh milk (g)	400	400	400
Whipping cream (g)	300	300	300
Sugar (g)	120	120	120
Pitaya pulp (%)	12	15	18
Stabilizer	10	10	10
Egg yolk (g)	80	80	80
Lime juice (mL)	5	5	5

Sensory analysis

Sensory evaluation for each ice cream sample was carried out with 20 semi-trained panelists after two days of preparation of ice cream on texture, flavor, appearance and overall acceptability, Flavor and color were determined during storage up to 16 weeks in biweekly intervals. The scores were analyzed by Friedman ranking test ($p<0.05$) and mean separation (Meilgaurd, 1999) using MINITAB 11.

Microbiological analysis

Total plate counts were determined in serial dilutions for each ice cream sample in weeks by using poured plate method (FDA, 2004).

Physical analysis

Complete melting time was measured according to Guven and Karaca (2002) method . 25 g of tempered samples were left to melt (at room temperature, 20°C) on a 0.2 cm wire mesh screen above a beaker and the complete melting time of the ice cream was determined. TSS of ice cream was estimated using a handheld refrectrometer (N-4E, Japan). The overrun of the final product was calculated using Eq. 1 as described in (Akin, 1990).

$$\text{Overrun} = \frac{\text{Weight of unit mix-weight of Equal volume of ice-cream}}{\text{Weight of equal volume of ice-cream}} \times 100\% \quad (1)$$

Chemical analysis

pH, titratable acidity, fat content and protein content of ice cream samples were measured using different laboratory techniques recommended by AOAC (1995). The samples were analyzed weekly for 16 weeks of storage at -14°C. The pH of ice cream was measured by using a laboratory pH meter and titratable acidity was measured by titrating 10.0 mL of filtered ice cream with 0.1 M NaOH using phenolphthalein as an indicator. The dry matter of milk samples was determined by exposing to 100±1°C for 3.5 h using an air oven (Akin, 1990), fat content in dry basis was determined by using soxlet method and for the estimation of protein, kjeldahl analysis was conducted on dry ice cream sample.

Results and discussion

The proximate compositions of pitaya pulp and pitaya incorporated ice cream is tabulated as below. The composition of fresh dragon fruit pulp such as moisture, TSS, reducing sugar, non-reducing sugar, total sugar, and ash, pH, acidity and vitamin C contents recorded in Table 2. The results obtained are in good agreement with those of ICBF (1992) and Morton (1987). The result of vitamin C content 10.90 mg/100g supported by Teddy (2008) who reported 9 mg/100 gm, beside this pH (4.20) and acidity (0.45) also closely related.

Table 2:
Sensory evaluation results - Mean scores given by panelists for different treatments with Standard deviation.

Character	p value	mean	±SD
Texture	0.005	5.48	0.718
Flavor	0.002	5.53	0.671
Appearance	0.000	5.58	1.041
Overall Acceptability	0.000	5.51	1.073

There was significant difference between the sensory characters ($p < 0.05$). When the pitaya pulp content was increased, the overall acceptability was reduced it may be due to the increased level of pectin present in pitaya pulp and the change in the texture of pitaya ice cream: "fig1". According to the sensory evaluation results, formula of 12% of pitaya pulp was selected as most acceptable for final product. Although the freezing temperature reduces the total microbial count to a lower level since the chemical reaction is continued, it cannot be completely stopped thus resulted the changes in pH, titratable acidity and brix value.

There was no significant difference ($p > 0.05$) between the pH of pitaya ice cream during storage time up to 12 weeks (table 4) But thereafter, the changes were significant. There was slight increase in the pH probably due to oxidation reaction of antioxidants, chemical and bio chemical mechanism (Hui, 2006).

Table 3:
Comparison of the composition of pitaya fruit pulp and pitaya incorporated ice cream

Parameters	Pitaya fruit pulp	Pitaya incorporated ice cream
Moisture (%)	81.20±0.05	60.42±0.04
Ash (%)	0.41±0.03	3.7±0.16
Reducing sugar (%)	4.20±0.06	*
Non-reducing sugar (%)	3.60±0.03	*
Total sugar (%)	7.80±0.01	*
TSS	10.5±0.02	31.5±0.01
pH	4.50±0.01	6.56 ±0.03
Titratable Acidity (%)	0.43±0.04	0.15±0.01
Vitamin-C (mg/100g)	8.30±0.04	*
Dry matter content (%)	11.80±0.04	39.58±0.28
Protein (%)	*	7.73±0.34
Fat (%)	*	45.37±0.99
Moisture (%)	81.20±0.05	60.42±0.04

*** Not Determined**

Table 4:
Chemical and physical characteristics of pitaya incorporated ice cream during the storage

Period of storage (Weeks)	Flavor	color	pH	Over run value (%)	Complete melting time(s)	Microbial count (log cfu/mL)	Remarks
0	fresh	Light yellow	6.56^{a}	22.84^{a}	4572 ± 20^{a}	4.75	Good
2	fresh	Light yellow	6.56^{a}	22.82^{a}	4585 ± 12^{a}	4.48	Good
4	fresh	Light yellow	6.55^{a}	22.79^{a}	4596 ± 22^{a}	4.23	Good
6	fresh	Light yellow	6.55^{a}	22.78^{a}	4610 ± 20^{a}	4.18	Good
8	fresh	Light yellow	6.55^{a}	22.76^{a}	4615 ± 31^{a}	3.62	Good
10	fresh	Light yellow	6.54^{a}	22.73^{a}	4635 ± 15^{b}	3.45	Good
12	fresh	Light yellow	6.54^{a}	22.54^{b}	4640 ± 16^{b}	3.26	Good
14	fresh	Light yellow	6.48^{b}	22.51^{b}	4648 ± 12^{b}	3.14	Good
16	off flavor	Light yellow	6.49^{b}	21.49^{b}	4679 ± 23^{c}	3.04	Not good

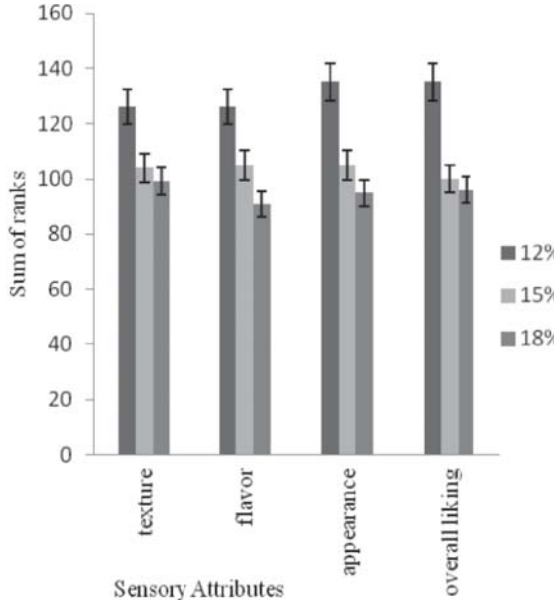
The values are means of three independent determinations. The means with different superscripts in a column differ significantly ($p < 0.05$).

Complete melting time was slightly reduced but there was no significant difference until 8 weeks but after that the changes were significance ($p<0.05$). After 14 weeks changes were higher. The brix value of pitaya ice cream was slightly changing with the storage time. In pitaya fruit malic acid is produced through the chemical reaction during storage period (Nomura et al, 2005) it may have led to the pectin to be hydrolyzed during storage (Diaz et al, 2007). There was no significant difference between the total soluble solid ($p>0.05$). It reveals that the shelf life of this product is confined to 3 months according to the physical and chemical characteristics (Table 4).

According to the Frozen Confections Regulation, any frozen confection for sale should not contain more than50, 000 bacteria per gram. According to the microbial evaluation for the pitaya ice cream sample, the initial microbial population was less than 50,000 /ml and it was in the acceptable microbial range. Even in the storage period of pitaya ice cream, there was only a slight reduction in bacterial counts. The decline in bacterial counts, as a result of freezing, was likely due to the freeze injury of cells, leading eventually to the death of cells. However, the mechanical stresses of the mixing and freezing process and also the incorporation of oxygen into the mix may have resulted in a further decrease in bacterial count. Similar results were reported by Ravula and Shah (1998); Haynes and Playne (2002); Mufas and Perera (2012).

In this study artificial colorings or preservatives were not added. The shelf life of the product may be increased by such additives. It is expected that the lime juice added, could provide some additional vitamin C to replace the loss during processing and storage of pitaya incorporated ice cream. Egg yolk is added for difference purposes: to provide light yellow color so it could be preferred by consumers, to provide higher whipping property to ice cream which could satisfy the overrun value and complete melting time of standard ice cream and to provide higher nutritional value because of its richness in some essential vitamins and minerals (Nau, 2010).

Fig. 1: Panelist preference for different sensory attributes



Conclusion

The proximate composition of pitaya fruit pulp and pitaya incorporated ice cream was found out. Study concluded the feasibility for valuable venture of producing ice cream using 12% of pitaya fruit pulp with higher acceptability to fulfill the market requirements. The shelf life of the pitaya incorporated ice-cream is three months. As the fruit has some special, medicinal and nutritive value (Taiwan Food Industry Development and Research Authorities, 2005), it is assumed that it could fetch a good economical value from the consumers. Therefore, the present study is a sign of bright prospect of processing of pitaya fruit into value added ice-cream. In this value addition process, the post harvest loss of pitaya is minimized. Further investigation is necessary to study the economic aspects of the products before recommending for commercial level.

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References

Abdul Azis Ariffin, Jamilah Bakar, Chin Ping Tan, Russly Abdul Rahman, Roselina Karim, and Chia Chun Loi. (2008). Essential fatty acids of pitaya (dragon fruit) seed oil. Food Chemistry, 114 (2): 561–564.

Akin, M.S. (1990). Effects of inulin and different sugar levels on viability of probiotic bacteria and the physical and sensory characteristics of probiotic fermented ice-cream. Harran University Agricultural Faculty Department of Food Engineering, sanliurfa, Turky, p.297-301.

AOAC. (1995). Official Methods of Analysis. Standard compendium of laboratory methods for analyzing foods and related substances. 16th ed. Washington DC, USA.

AOAC. 2004. Official Methods of Analysis. Association of Official Analytical Chemists, Washington DC, USA.

Arevalo-Galarza, M. de l. and Ortiuz-Herrnandez, Y.D. (2004). Postharvest behavior of pitaya fruit (*Hylocereus undatus*). Cactaceas-Succulentas-Mexicanas 49 (3): 85-90.

Brody, T., (1994) Nutritional Biochemistry; Academic Press: San Diego, CA; pp. x and 450-9.

Diaz, J.V., Anthon, G.E., Barrett, D. M. (2007). Non enzymatic Degradation of Citrus Pectin and Pectate during Prolonged Heating: Effects of pH, Temperature, and Degree of Methyl Esterification. Department of Food Science and Technology, University of California, Davis, California 95616.

Food and drug administration (2004) Bacteriological analytical manual, Chapter 9.

Gunasena, H.P.M., Pushpakumara, D.K.N.G and Kariyawasam, M. (2006) Dragon fruit-Hylocerus undatus (Haw.) Britton and rose: field manual for extension workers. Sri Lanka Council for agricultural Policy, Wijerama Mawatha, Colombo 7, Sri Lanka.

Guven, M., Karaca, O. B. (2002). The effect of varying sugar content and fruit concentration on the physical properties of vanilla and fruit ice cream type frozen yoghurt. *International journal of dairy technology*.55, 27-31.

Haynes, I. N., & Playne, M. J. (2002). Survival of probiotic cultures in low-fat ice-cream. *Australian Journal of Dairy Technology*, 57, 10–14.

Hui. Y.H., Chen.F. And Nollet.L.M.L (2006). Hand Book of Fruit and Vegetable Flavor. John willey and sons.Inc. Hoboken, Newjersy, USA.

ICBF (1992). Table de Composicion de Alimentos. 6th Ed. From: El cultivo de Pitaya Fposicionmiento en elmercardo.http://www.worldagroforestry.org/downloads/publications/PDFs/BC07324.PD, 18 February 2011.

Meilgaurd, M., Civille,C.V., and Carr, B.T. (1999) Sensory Evaluation Techniques, CRC, Boca Raton, FL.

Mizrahi, Y. and Nerd, A. (1999). Climbing and columnar cacti: New arid land fruit crops. Progress in new crops. ASHS Press Alexandria, VA. 56-64 pp.

Morton, J. F. 1987. Fruits of Warm Climates, Strawberry Pear. Florida Flair Books, Miami, 347-348 and505 pp.

Mufas A.H.M., Perera O.D.A.N. (2012) Alteration of pitaya pulp into a value added product to reduce the post harvest losses. International research conference, University of Jaffna, Sri Lanka, ISSN: 2279-1922, page: 193.

Nau F, Nys Y, Yamakawa Y, Rehault-Godbert S (2010). Nutritional value of the hen egg for humans. Inra Prod.Anim., 23: 225-236.

Nerd, A. Tel-Zur, N. and Mizrahi, Y. (2002). Chapter 10: Cactus Pear Fruit Production In: Nobel, P.S. (ed.) Cacti: biology and uses. University of California Press, Berkeley, 163 p.

Nobel, P. S. and de la Berrera, E. 2002. Stem water relations and wet C02 uptake for hemiepiphytic cactus during short term drough. *Environment and Experimental Botany*, 48: 129-137.

Nomura, E.S. (2008), Crescimento de mudas micropropagadas da bananeira cv. Nanicao, em diferentes substratos e fontes de fertilizante. Acta Sci. Agron., Maringa, v. 30, n. 3, p. 359-363.

Ravula, R. R., & Shah, N. P. (1998). Effect of acid casein hydrolysate and cysteine on the viability of yogurt and probiotic bacteria in fermented frozen dairy desserts. *Australian Journal of Dairy Technology*, 53,175–179.

Taiwan Food Industry Develop and Research Authorities (2005).

Teddy, F. Tepora. 2008. Program Leader, CvSU R&D on Dragon Fruit. Research Center, Cavite State University. Philippines. -fruit-enter-the-dragon/17 Sep 2008.

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Influence of effective microorganisms on root-shoot ratio and harvest index of groundnut *(Arachis hypogaea L.)*

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Abstract: Effective microorganisms (EM) solution was used in combination with different rates of cattle manure to study feasibility of EM in groundnut cultivation. This study determined the fraction of biomass partitioned to root vs. shoot in groundnut response to cattle manure and EM application. Significant reduction in root- shoot ratio was noted with manure application. Further, it was observed that EM application significantly improved harvest index (HI) of the crop. It suggests that cattle manure at the rate of 15 t/ha with EM would give better plant performance of groundnut.

Keywords: Cattle manure, Effective microorganism, groundnut, R-S ratio, harvest index

Introduction

The relative growth rates of root and shoot are important parameters of dry matter production (Cannell and Willett, 1976). The root-shoot ratio (R-S) is given as the ratio of dry weight of the roots to the dry weight of the top of a plant (Harris, 1992). R-S ratio correlates with inherent factors such as plant species, age and region (Mokany *et al*., 2006). For most crop plants, except tuber crops R-S ratio is high early in the growing season and decrease with crop maturity. R-S ratio of root and tuber crop (where root is sink) increases with maturity of crop and favorable conditions for tuberization also increase the R-S ratio in tuber crops (Rogers *et al*., 1996). R- S ratios of adult plants in Mediterranean ecosystems to be higher than temperate ecosystems, possibly as an adaptation to dry season (Hilbert and Canadell 1995).

The R-S ratio is one of the growth parameter to assess the performance of crops and overall health of plants. R-S ratio depends upon the partitioning of photosynthate which may be influenced by the external factors. There are studies shows that R-S of crops influenced by nutrients, water deficient, carbon dioxide concentration (Lindquist *et al*., 2005; Cakmak *et al*., 1994; Gutschick, 1993; Rogers *et al*., 1996). Cattle manure is commonly used organic manure and use of effective microorganism is also expanding in many countries. Studies showed that EM has positive effect on crop performance in organic farming system (Sangakkara and Higa, 1992; Sangakkara, 1994; Sangakkara, 1996). Groundnut (*Arachis hypogaea* L.) is commonly grown leguminous crop in Sri Lanka and it showed positive yield response to organic manure (Chandrasekaran *et al*., 2000).The aim of this experiment was to determine the response of R-S ratio and HI to EM in groundnut (*Arachis hypogaea* L.) when applied with cattle manure.

Material and Methods

The experiment was conducted in 2010 at the Agronomy farm of Eastern University of Sri Lanka. The experimental site comes under the agro ecological zone of low country dry zone [7° 43'N, 81° 42'E]. The soil is sandy regosol. Treatments included presence (T_1) and absence (T_2) of the recommended dose of

inorganic fertilizer and different levels (5, 10, 15 and 20 tha^{-1}) of cattle manure with or without EM soil application (T_3-T_{10}) as indicated in Tab 1. All treatments were replicated four times in Randomized Complete Block Design. Cattle manure (CM) was applied in different rates to plots (T_3-T_{10}) two weeks before sowing. Planting space of groundnut was 45 cm between rows and 15 cm within a row with one seed per hole and each plot measured 2.2 x 2.0 m. EM solution was prepared two hours before spraying by diluting EM stock solution with molasses and water (1:1:1000) as recommended in EM application manual (Anon., 1995). The EM solution was sprayed in two weeks interval from 4^{th} week onwards upto 10^{th} week of planting groundnut at the rate of 10L/ha.

All other agronomic practices except fertilizer management were followed according to the recommendation. Observations were made on plant height, number of branches and dry weight of stem, root and pods in each treatment at the time of harvesting. Plant parts i.e stem, pods and root in each treatment were oven dried at 105°C over night to determine the dry weights and R-S ratios were calculated. In a physiological perspective, R-S ratios have been interpreted as reflecting the differential investment of photosynthates between the aboveground and belowground organs (Titlyanova *et al.,* 1999). Additionally, Harvest index was also calculated. Harvest index was calculated by using weights of pods and total dry matter per plant. Collected data were statistically analyzed, significant difference between the treatments were determined using analysis of variance (ANOVA) using SAS software and the mean separation were done using Tukey's studentized range test at 5% level.

Table 1:
Treatment of study

Treatments	Code
With fertilizer (control)	T_1
No fertilizer + no CM	T_2
5 t/ha CM	T_3
5 t/ha CM + EM	T_4
10 t/ha CM	T_5
10 t/ha CM + EM	T_6
15 t/ha CM	T_7
15 t/ha CM + EM	T_8
20 t/ha CM	T_9
20 t/ha CM + EM	T_{10}

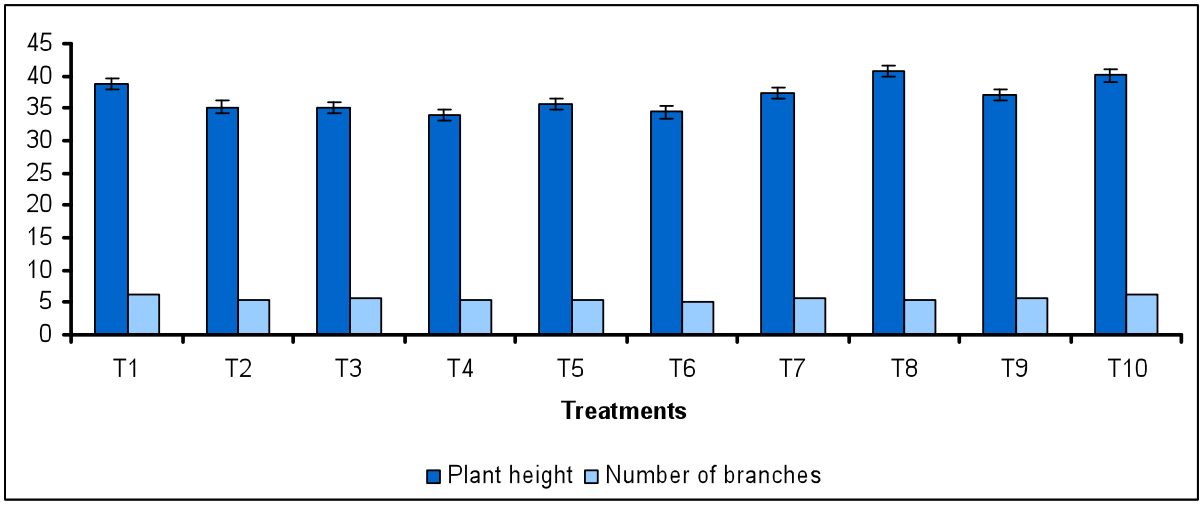

Figure 1: Plant height (cm) and number of branches per plant in each treatment at harvest

Results and Discussion

Plant height

There was significant difference (P<0.01) in plant height among the treatments (Fig 1). The difference in plant height among the treatments might be due to the variation in rate of manure application and EM combination. The average plant height ranges from 33.92 to 40.75cm. Maximum number of branches was observed in T_1. EM enhances the microbial diversity in soil (Higa and Parr, 1994) and thus improves soil health (Xu, 2000). The adequate absorption of essential nutrients requires for biochemical and physiological processes of plants (Ihejirika, 2007). Yousaf *et al* (2000) reported that EM inoculation improves the plant height in groundnut. Even though, number of branches per plant were not significantly differ (P>0.05) among the treatments. Branching habit is one heritable trait of crop.

Shoot-root ratio

Shoot dry weight showed significant differences (P<0.01) among treatments (Tab2). Increasing trend in dry weight of shoot was observed with increasing rate of CM application. Addition of CM may improve in soil health thus leading to high uptake of major nutrients resulted in better performance. CM contains considerable amount of nitrogen (N) and phosphorus (P) (0.35% N, 0.12% P_2O_5). N and P are primarily stimulating shoot and root growth respectively (Harris, 1992). Photosynthetic microorganisms are one constituent of EM which enhances photosynthesis (Wang *et al.*, 2000) and it may lead to high dry matter accumulation in plant parts. It was reported that increase in cattle manure rate increase the total dry matter yield per plant in okra (Dada and Fayinminnu, 2010).

Results shown in Tab 2 revealed that R-S ratio of crop was altered either by manure application or EM. The mean R-S ratio ranges from 0.061 to 0.112.Comparatively higher R-S ratios were obtained in T_2 and T_3 than other treatments and the values are significantly differed (p<0.01) from all others treatments. The nutrients content in T_2 and T_3 were low. It was clear that increase in nutrient content of soil either by chemical fertilizer or optimum amount (>10 t/ha) of organic manure leads reduction in R-S ratio of crops. Further it was noticed that R-S ratio in T_3 was significantly (p<0.05) differed from T_4 which implied that EM application has indirect influence on R-S ratio. A reduction in R-S ratio is in response to more favourable growing condition (Harris, 1992; Rogers *et al.*, 1996). However, gradual increase in manure rate had no significant (p>0.05) influence on R-S ratio of groundnut. It is probably because of the ability of plants to adapt to changing conditions when the changes are not too drastic or rapid (Harris 1992).

Table 2: The R-S ratios of groundnut in each treatment

Treat ments	Dry weight of shoot / plant (g)	Dry weight of root /plant (g)	R- S ratio
T_1	40.37 ± 2.26 abc	2.66 ± 0.35	0.066 b
T_2	24.86 ± 1.04 e	2.74 ± 0.16	0.110 a
T_3	26.98 ± 0.96 de	3.04 ± 0.45	0.112 a
T_4	31.50 ± 1.20 cde	2.34 ± 0.17	0.073 b
T_5	32.04 ± 1.18 cde	2.14 ± 0.04	0.067 b
T_6	36.44 ± 2.03 bcd	2.22 ± 0.10	0.061 b
T_7	39.16 ± 2.92 abc	2.57 ± 0.16	0.066 b
T_8	44.84 ± 1.78 ab	2.79 ± 0.13	0.062 b
T_9	42.27 ± 1.16 ab	2.68 ± 0.18	0.063 b
T_{10}	47.59 ± 3.22 a	2.99 ± 0.39	0.062 b
F value	**	ns	**
CV%	10.97	21.91	16.7

Table 3: The influence of cattle manure with EM on plant dry matter and harvest index of groundnut

Treat ments	Crop residue /plant (g)	Dry weight of pods /plant (g)	Harvest index
T1	19.52 ± 1.55 ab	23.52 ± 1.32 ab	0.54 ± 0.02 a
T2	15.89 ± 0.25 b	11.70 ± 0.88 d	0.42 ± 0.01 c
T3	16.58 ± 1.11 ab	13.44 ± 0.82 d	0.45 ± 0.02 bc
T4	16.49 ± 0.76 ab	17.34 ± 0.73 c	0.52 ± 0.01 ab
T5	15.95 ± 0.50 bc	18.22 ± 0.92 c	0.53 ± 0.01 a
T6	17.16 ± 0.59 b	21.50 ± 1.65 bc	0.56 ± 0.02 a
T7	19.22 ± 1.58 ab	22.50 ± 1.55 b	0.54 ± 0.01 a
T8	22.51 ± 1.68 a	26.63 ± 1.22 a	0.53 ± 0.01 ab
T9	20.60 ± 0.92 ab	23.85 ± 0.91 ab	0.54 ± 0.01 a
T10	22.36 ± 2.45 a	26.22 ± 1.60 a	0.56 ± 0.02 a
F value	*	**	**
CV%	**14.1**	**12.3**	**6.6**

Value represents mean ± standard error of four replicates. F test: - **: P< 0.01.ns: not significant. Means followed by the same letter are not significantly different according to Tukey's studentized range test Duncan's Multiple Range Test at 5% level

Pod yield and Harvest index

EM application showed significant influence (P<0.01) on crop residue and pod yield of groundnut fertilized with cattle manure (Tab 3). Crop residue is the sum of dry matter accumulated in plant parts except economic parts. The average dry matter (oven dry weight) of crop residue and pods per plant ranged from 15.89 g to 22.51 g and 11.70 g to 26.63 g respectively. Pods are the sink for dry matter accumulation in groundnut therefore highly significant variation (P<0.01) exhibited in pod dry matter at harvest among the treatments. The highest dry matter (both crop residue and pods) was obtained in T_8 (15 t/ha CM with EM) and it was statistically similar with the pod yield of chemical fertilizer (T_1). Javaid and Mahmood (2010) stated that EM application significantly enhanced shoot and pod biomass of soybean with farmyard manure application.

Result showed that there was significant (P<0.01) effect on harvest index (HI) due to the manure application (Table 3). It was revealed that to achieve high HI (more than 0.5), the plant required to fertilize with optimum level of manure. The average value of HI ranged from 0.42 (T_2) to 0.56 (T_8). Harvest index indicates the partitioning of photosynthate between economic plant part and crop residue (vegetative plant parts) of groundnut. HI values for peanuts ranged from 20% to 47% (Fageria *et al.,* 2006).

Conclusion

In this study, the results indicated that combination of EM with cattle manure had significant improvement in plant growth. Manure application had significant reduction in R-S ratios of groundnut. Further, it was noticed that EM significantly improved harvest index of crop. Cattle manure (15 t/ha) with EM would give better plant performance on sandy regosol.

References

Anon. (1995). EM application manual for APNAN countries 1st edition. Asia-Pacific Natural Agriculture Network, Bangkok, Thailand. Access on 12.10.2011 from

Chandrasekaran, R., E. Somasundaram, A.M. Mohamed, K. Nalini, K. Thirukkumaran and K. Sathyamoorthi, (2007). Response of confectionery groundnut (*Arachis hypogaea* L.) varieties to farm yard manure. J. Applied Sci. Res. 3(10): 1097-1099.

Cakmak, I., Hengeler, C and Marschner, H (1994). Partitioning of shoot and root dry matter and carbohydrates in bean plants suffering from phophours, potassium and magnesium deficiency. *J. Exp. Bot*. 45:1245-1250.

Cannell, M. G. R and Willett, S.C (1976). Shoot growth phenology, Dry matter distribution and Root: Shoot ratios of provenances of Populus trichocarpa, Picea sitchenis and Pinus contorta growing in Scotland, *Silvae Genetica* 25(2): 49-58.

Dada, O. A and Fayinminnu, O. O (2010). Period of weed control in okra (*Abelmoschus esculentus* L.) Moench] as influenced by varying rates of cattle dung and weeding regimes, *Notulae Botanicae Horti AgrobotanicCluj-Napoca* 38 (1):149-154.

Gutschick V. P (1993) Nutrients-limiting growth rates: role nutrient use efficiency and of adaptations to increase uptake rate. *Journal of Experimental Botany*.44: 41-51

Fageria, N.K.., Baligar., V.C.B and Clark., R. B (2006). Physiology of crop production Food products Press, New York, pp 73-74.

Harris, R.W (1992). Root-Shoot ratios. *Journal of Arboriculture* 18(1): 39-42

Higa, T and Parr, J.F (1994). Beneficial and Effective microorganisms for a sustainable Agriculture and Environment, Bulletin, International Nature Farming Research Center, Atami, Japan (http://www.agriton.nl/higa.html)

Hilbert, D.W. and Canadell, J. (1995). Biomass partioning and resource allocation of plants from Mediterranean-type ecosystems: possible responses to elevated atmospheric CO2. In Moreno, J. M and Oechel, W. C. (eds) Global Change and Mediterranean-Type Ecosystems pp. Springer-Verlag, New York. Pp: 76–101.

Ihejirika G.O (2007). Evaluation of organic manures and plant densities on pod rot, nodulation and seed weight of groundnut in an Ultisols. *Journal of Plant Science*, 2: 475-479.

Javaid, A and Mahmood, N (2010). Growth, nodulation and yield response of soyabean to biofertilizer and organic manures. P. J. Bot., 42 (2): 863-871.

Lindquist, J. L., Bonifas, K.D., Walters, D.T and Cassman, K. G (2005). Nitrogen supply affects root: shoot ratio in corn and velvetleaf (Abutilon theophrasti), *Weed Science,* 53:670–675

Mokany, K., Raison, R.J and Prokushkin, A.S (2006). Critical analysis of root : shoot ratios in terrestrial biomes. *Global Change Biology* 12: 84–96

Rogers, H.H., Prior, S.A., Runion,G.B and Mitchell, R.J (1996). Root to shoot ratio of crops as influenced by CO_2. *Plant and soil* 187: 229-248

Sangakkara, U. R (1994). Effect of EM on growth and yield of sweet potato in wet and dry seasons. P103-110. In Parr J. F., S. B Hornick and M. E Simpon (ed) Proceeding of the 2rd International Conference on Kyusei Nature Farming U.S Department of Agriculture, Washington DC , USA

Sangakkara, U. R (1996). Effect of EM on nitrogen and potassium levels in the rhizosphere of bush bean pp 216-222 In Proceeding of the 3rd International Conference on Kyusei Nature Farming. U.S Department of Agriculture, Washington DC , USA

Sangakkara, U. R and T. Higa. (1992). Effective Microorganism for organic agriculture: A case study from Sri Lanka. P 152-159 In U. Kope and U. Schitz (ed) Proceeding of the 9th IFOAM Conference. Tholey-Theloy Germany.

Titlyanova A. A., Romanova I.P., Kosykh, N.P (1999). Pattern and process in above-ground and below-ground components of grassland ecosystems. *Journal of Vegetation Science*, 10: 307–320.

Wang, R., Xu, H.L. and Mridha, M.A.U (2000). Effect of Organic Fertilizer and EM Inoculation on Leaf Photosynthesis and Fruit Yield and Quality of Tomato Plants. *Journal of Crop Production*, 3(1): 173-182.

Xu, H.L. (2000). Effect of a Microbial Inoculant, and Organic Fertilizer, on the Growth, Photosynthesis and Yield of Sweet Com. Journal of Crop Production, 3(1): 183-214.

Yousaf, Z., Jilani., G., Qureshi, R.A and Awan, A.G. (2000). Effect of EM on Groundnut (*Arachis hypogea* L.) growth. *Pakistan Journal of Biological Science* 3(11): 1803-1804.

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Effect of EM-Bokashi on vigorous seed production in vegetable cowpea *(Vigna unguiculata L.)*

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Abstract: The experiment was conducted to study the effect of EM-Bokashi on seed performance in vegetable cowpea (*Vigna unguiculata* L.). Results showed that the seeds harvested from animal manure EM-bokashi recorded significant increases in their vegetative and reproductive growth parameters than those from chemical and non-fertilized plants. Among the seeds harvested from different types of animal manure Bokashi, seeds in goat manure EM-bokashi showed high dry weights of stem, number of nodules, pod weight and plant biomass. Hence, it could be concluded that the use of animal manure with EM in vegetable cowpea cultivation could give the healthy seeds as planting material for obtaining high pod and seed yields and also for favourable health and environment.

Key words: Animal manure, effective microorganism, vegetable cowpea seeds, sandy regosol

Introduction

Vegetable cowpea (*Vigna unguiculata*) is widely cultivated in the Eastern region of Sri Lanka and other Asian countries. In conventional agriculture, chemical fertilizers are an important component however in recent years the safety and health of food has becoming a major concern due to overuse of chemicals for food production and its negative impacts on human health and environment (Pimentel, 2005). Most agricultural practices affect soil quality by altering the soil properties and have led to a decrease in soil microbial populations resulting in decreased crop productivity (Valarini *et al*., 2002). Therefore, organic materials hold great promise as a source of multiple nutrients and ability to improve soil characteristics (Moller, 2009). Since the effect of organic nutrients on crop yield is long term and not immediate, farmers are reluctant to use organic fertilizers in their cropping system and use of EM (effective microorganisms) along with organic materials possibly be an effective technique for stimulating release of nutrients from organic sources (Javaid, 2009). Application of EM is known to enhance crop growth and yield in many crops, both leguminous and non-leguminous (Sheng and Lian, 2002; Javaid, 2006, 2009; Khaliq *et al.,* 2006; Daiss *et al.,* 2008) High quality seed is a major factor in obtaining a better crop growth and their development even under adverse conditions (FAO, 2007). This experiment was aimed to study the influence of EM-Bokashi on vigorous seed formation for crop growth of vegetable cowpea on sandy regosol.

Materials and Methods

In the previous experiment, the vegetable cowpea plants *cv. Bushitao* were grown in non-fertilizer, inorganic fertilizer and also three different types of fermented organic matters with EM. Subsequently seeds were obtained and used in this study to evaluate the seed performance of vegetable cowpea. The present experiment was carried out in the Eastern region of Sri Lanka in 2010/2011. Inorganic fertilizer recommended by the Department of Agriculture was applied for vegetable cowpea cultivation. The manure fermented with EM (EM-Bokashi) was separately made from rice bran, rice husk and animal manure (cattle manure, goat manure and poultry manure) at the ratio of 1:1:2 (w/w/w). They were mixed together and added EM solution. EM-bokashi (300 g/m^2) was applied as basal

and top dressing as recommended by kyan *et al.* (1999).

The collected seeds from the previous experiment as mentioned above were planted at 60 cm x 20 cm spacing in the field without any fertilizer application (Table 1). The treatments were assigned in a randomized complete block design with five blocks. The other agronomic practices were done as recommended. The growth measurements in each plot (2.6 m^2) were taken at two weeks intervals. All the plant samples were oven dried at 105 ^oC, subsequently their dry weights were taken. Data collected were analyzed using SAS statistical computer package and treatment means were compared by using Tukey's Studentized Range (HSD) Test at 5% level.

Table 1: The seeds obtained from the previous experiment.

Treatments	Seeds harvested from the plants grown in different fertilizer regimes
T1 (control)	Non fertilizer application
T2	Chemical fertilizer application
T3	Cattle manure EM-bokashi application
T4	Goat manure EM-bokashi application
T5	Poultry manure EM-bokashi application

Results and Discussion

Leaf weight per plant

The highest dry weight of leaves was obtained in T4 treatment at the different growing periods (Table 2) and also the results showed that seeds from the animal manure EM-bokashi treatments (T3-T5) had higher value than the chemical and non-fertilizer (T2 and T1). There were significant differences among the treatments on dry weight of leaves (Table 2). Both T4 and T5 generated heavier leaf dry matter than the other treatments throughout the plant life. However, among the all treatments, seeds from goat manures EM-bokashi (T4) showed highest value at different growing periods. This may due to combination of goat and EM able to increase higher rate of growth and photosynthetic activity in leaf. In the present study, the seeds from animal manure EM-bokashi showed no significant different in leaf area among the treatments at the 4th week, but the following week interval, T4 showed significant ($P < 0.05$) difference than the other treatments. The result is supported with Hsieh and Hsieh (1990) who reported that goat manure EM-bokashi had high mean leaf area than other EM-bokashi due to having higher amount of potassium than the other treatments. Potassium compounds are important in plant nutrition because they have a marked influence upon the development of leaves (Lucius, 2001).

Table 2:
Effect of the source of seeds on leaf dry weight of vegetable cowpea.

	Vegetative stage		Flowering stage	Maturity stage
Treatments	**2 week**	**4 week**	**6 week**	**10 week**
T1 (control)	0.18 ± 0.01b	0.73 ± 0.06b	07.85 ±0.02b	06.95 ± 0.29b
T2	0.19 ± 0.01b	1.05 ± 0.02a	08.06 ± 0.38b	08.95 ± 0.20ab
T3	0.19 ± 0.01b	1.28 ± 0.06a	08.21 ± 0.37b	09.00 ± 0.56ab
T4	0.26 ± 0.01a	1.31 ± 0.06a	10.29 ± 0.48a	10.66 ± 0.47a
T5	0.23 ± 0.01ab	1.29 ± 0.01a	09.56 ± 0.33ab	09.15 ± 0.38a
F test	*	*	*	*
CV%	9.23	8.44	7.84	8.42

Values represent the mean ± standard error. F test: *- $P < 0.05$. Means with the same letter in each column are not significantly different at 5% level, according to Tukey's Studentized Range (HSD) Test.

Stem weight per plant

Result revealed that weight of stem per plant was increased in all the treatments compared to the control treatment (Table 3) and T5 showed high fresh weight among all the treatments at the 10th week. T4 had the high values in dry weight at the different stages except maturity stage. This was similar to the finding of Javaid *et al.* (2000) who found that the stem fresh weight was enhanced by EM application with any farm yard manures.

Table 3:
Effect of the source of seeds on stem dry weight of vegetable cowpea.

	Vegetative stage		Flowering stage	Maturity stage
Treatments	**2 week**	**4 week**	**6 week**	**10 week**
T1 (control)	0.082 ± 0.006c	0.316 ± 0.01b	6.28 ± 0.51	07.01 ± 0.52c
T2	0.089 ± 0.003bc	0.433 ± 0.03ab	7.12 ± 0.39	11.32 ± 0.64b
T3	0.094 ± 0.004bc	0.530 ± 0.05a	7.34 ± 0.09	12.56 ± 0.42b
T4	0.107 ± 0.003a	0.583 ± 0.02a	7.97 ± 0.28	18.25 ± 0.82a
T5	0.101 ± 0.004b	0.540 ± 0.02ab	7.75 ± 0.41	18.48 ± 0.95a
F test	*	*	ns	*
CV%	8.32	12.57	8.39	8.23

Values represent the mean ± standard error. F test: ns –P > 0.05;*- P < 0.05, **-P < 0.01 Means with the same letter in each column are not significantly different at 5% level, according to Tukey's Studentized Range (HSD) Test.

Number of nodules per plant

Number of nodules per plant in T4 was considerably higher compared with other treatments throughout life cycle of the plant (Table 4). This may be due to high amount of potassium present in goat manure. This result agrees with Lucius (2001) who found that potassium stimulates the formation of carbohydrates in the nodules and thereby makes them better fitted to support the nitrifying bacteria.

Table 4:
Effect of the different treatments on nodulation of vegetable cowpea.

	Number of nodules per plant			
Treatments	**2 week**	**4 week**	**6 week**	**10 week**
T1 (control)	1.33 ± 0.33b	07.00 ± 0.57d	17.00 ± 2.00b	09.33 ± 0.33b
T2	2.67 ± 0.33ab	08.00 ± 0.57d	19.33 ± 0.66b	10.67 ± 0.66ab
T3	3.67 ± 0.33a	14.00 ± 0.57b	20.33 ± 0.33b	13.67 ± 0.66a
T4	4.00 ± 0.57a	19.33 ± 0.33a	28.33 ± 0.66a	14.00 ± 1.00a
T5	2.67 ± 0.33ab	11.67 ± 0.33c	20.33 ± 0.33b	13.00 ± 1.00ab
F test	*	**	**	*
CV%	23.83	5.69	8.71	11.46

Values represent the mean ± standard error. F test: * -P < 0.05;**- P < 0.01. Means with the same letter in each column are not significantly different at 5% level, according to Tukey's Studentized Range (HSD) Test.

Root weight per plant

EM combined with animal manures treated seed's plants increased weight of root per plant (Table 5) than other treatments and goat manure EM-bokashi had high value. The effect of dry weight on different treatments and there were significant ($P < 0.05$) different among the treatments at the 2nd and 4th weeks thereafter no remarkable variation. Inoculation of effective microorganism can increase the available nutrition for plant roots and improve photosynthesis (Muthaura, 2010).

Table 5:
Effect of the different treatments on root dry weight of vegetable cowpea

	Vegetative stage		Flowering stage	Maturity stage
Treatments	**2 week**	**4 week**	**6 week**	**10 week**
T1 (control)	0.031 ± 0.004b	0.15 ± 0.03b	1.19 ± 0.27	1.62 ± 0.38
T2	0.045 ± 0.003ab	0.22 ± 0.03b	1.37 ± 0.16	1.88 ± 0.46
T3	0.054 ± 0.003ab	0.26 ± 0.02ab	1.59 ± 0.31	2.34 ± 0.45
T4	0.059 ± 0.002a	0.35 ± 0.016a	1.69 ± 0.05	2.51 ± 0.45
T5	0.051 ± 0.003ab	0.20 ± 0.03b	1.51 ± 0.02	2.40 ± 0.42
F test	*	*	ns	ns
CV%	12.81	19.17	25.05	28.49

Values represent the mean ± standard error. F test: ns –$P > 0.05$;*- $P < 0.05$. Means with the same letter in each column are not significantly different at 5% level, according to Tukey's Studentized Range (HSD) Test.

Pod weight

Significant variations in pod length as well as dry weights of green pod were observed among the treatments (Table 6). EM increased the pod number and weight as reported by Hussain *et al.* (1994). The present result is due to seed sources of vegetable cowpea that could improve plant growth and yield. The seeds collected from plants fertilized with animal manure EM-Bokashi, exhibited better plant performance.

Table 6:
Effect of the different treatments on green pod and plant biomass

Treatments	**Pod length (cm)**	**Pod fresh weight (g)**	**Pod dry weight (g)**	**Plant biomass (g)**
T1 (control)	21.20 ± 0.29b	19.82 ± 0.23d	1.68 ± 0.02b	36.29 d
T2	22.58 ± 0.22ab	23.45 ± 0.25b	2.18 ± 0.29ab	59.21 c
T3	22.15 ± 0.96b	21.41 ± 0.49c	2.52 ± 0.49b	77.65 b
T4	24.72 ± 0.45a	25.10 ± 0.45a	3.01 ± 0.20a	101.64 a
T5	23.12 ± 0.54ab	22.86 ± 0.46bc	2.62 ± 0.39ab	85.91 b
F test	*	**	*	*
CV%	3.69	2.35	16.84	13.21

Values represent the mean ± standard error. F test: * -$P < 0.05$;**- $P < 0.01$. Means with the same letter in each column are not significantly different at 5% level, according to Tukey's Studentized Range (HSD) Test.

Plant biomass

The effect of source of seeds on plant biomass is showed in Table 6 and there was significant difference ($P<0.05$) on plant biomass among the treatments. The seeds collected from plant grown in goat manure EM Bokashi exhibited significantly ($P < 0.05$) high plant biomass and pod yield followed by seeds from poultry manure. This finding is supported by Yan and Xu (2002) who reported that the pod dry weight of peanut in EM-bokashi fertilizer treatment was significantly higher than that in chemical fertilizer treatment. EM application has proved beneficial in increasing crop growth and yield in mungbean and vegetables (Sangakkara and Higa, 1994). The present results also indicated that seeds collected from plant grown in EM-bokashi treatments expressed more plant biomass than chemical and non-fertilizer application treatments. Plant biomass is an important parameter that influences on yield of the crop especially legumes that are grown for food, feed and green manuring. The results revealed that seeds collected from plants grown in EM-bokashi treatments remarkably showed high ($P<0.05$) plant biomass than those in chemical and non-fertilizer treatments and also seeds from goat manure with EM-bokashi significantly ($P<0.05$) enhanced biological yield among the seeds harvested from different types of animal manure EM-Bokashi treatments.

Conclusion

Application of animal manure treated with EM solution especially goat manure-EM-bokashi as a substitute of inorganic fertilizers gave the healthy seeds as planting material. The vigorous seeds are a major factor in obtaining a good crop growth and their development ultimately achieves optimal biological and economic yields. Therefore, this organic cultural practice as a substitute of inorganic cultivation can be adapted by farmers for producing good crop with lesser health and environmental problems.

References

Daiss, N., Lobo, M.G., Socorro, R., Bruckner, U., Heller, J. and M. Gonzaler (2008). The effect of three organic pre-harvest treatments on Swiss chard (*Beta vulgaris* L. var. *cycla* L.) quality. Eur. Food Res. Technol., 226: 345-353.

Hsieh, S.C. and Hsieh, C.F. (1990). The use of organic matter in crop production. Taiwan, ROC: Taichung District Agricultural Improvement Station, 315**:** 1-19.

Hussain, T., Ahmad, R., Jilani, G. M. and Higa, T. (1994). Applied EM Technology. Nature Farm. Res. Center, Univ. Agri., Faisalabad, Pakistan, pp: 1-6.

Javaid, A., Bajwa, R., Rabbani, N. and Uzma, M. (2000). EM and VAM Technology in Pakistan IX: Effect of EM application on growth, yield, nodulation and VA Mycorrihizol colonization in *Vigna radiate* (L.) Wiczex. Pakistan J. Biol. Sci., 3(4): 694-698.

Javaid, A. (2006). Foliar application of effective microorganisms on peaas an alternative fertilizer. Agron. Sustain. Dev., 26: 257-262.

Javaid, A. (2009). Growth, nodulation and yield of black gram [*Vignamungo* (L.) Hepper] as influenced by biofertilizers and soil amendments. African Journal of Biotechnology 8 (21): 5711-5717.

Khaliq, A., Abbasi, M.K. and T. Hussain. (2006). Effect of integerated use of organic and inorganic nutrient sources with effective microorganisms (EM) on seed cotton yield in Pakistan. *Bioresour. Technol.,* 97: 967-972.

Kyan, T. Shintani, M. Kanda, S. Sakurai, M. Ohashi, H. Fujisawa, A. and Pongdit, S. (1999). Kyusei nature farming and the technology of effective micro

organisms. International Nature Farming Research Center (INFRC), Atami, Japan and Asia Pacific Natural Agriculture Network (APNAN), Bangkok, Thailand.

Lucius L. V. S. (2001). Fertilizers and crop production. Updesh purohit for Agrobios, Joodhpur, India.

Moller K (2009). Influence of different manuring systems with and without biogas digestion on soil organic matter and nitrogen inputs, flows and budgets in organic cropping systems. Nutr. Cycling Agroecosyst. 84: 179-202.

Muthaura, C., Musyimi, D.M., Ogur, J. A and Okello, S. V. (2010). Effective microorganisms and their influence on growth and yield of Pigweed (*Amaranthus dubians*). Asian Research Publishing Network (ARPN) Kenya . J. Agric.Biol. Sci., 5 (1):17-22.

Pimentel, D., Hepperly, P. Hanson, J. Douds, D. and Seidel, R. (2005): Environmental, energetic, and economic comparisons of organic and conventional farming systems. BioScience. 573:582.

Sangakkara, U.R. and Higa, T. (1994). Effect of EM on nitrogen fixation by Bush bean and Mungbean.In: *proc. 2nd. Int.Conf., Kyusei Nature farming*, 7-11 October, 1991, Brasil. pp:111-117.

Sheng, Y.P. and Lian. X.H. (2002). Influence of EM Bokashi on nodulation, physiological characters and yield of peanut in nature farming fields. *J. Sustain. Agric.,* 19: 105-112.

Valarin, P.J., Alveraz, M.C.D,, Gasco, J.M, Guerrero, F. and Tokeshi, H. (2002). Integrated evaluation of soil quality after the incorporation of organic matter and micro-organisms. Braz. J. Micr., 33: 35 – 40.

Yan Pei-Sheng and Xu Hui-Lian. (2002). Influence of EM Bokashi on Nodulation, Physiological Characters and Yield of Peanut in Nature Farming Fields. J. , 19(4)**:** 105-112.

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Physical, textural and sensory attributes of plain set yoghurt made employing ultrafiltration technique as affected by titratable acidity during incubation

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Abstract: Effect of stopping incubation at different titratable acidity levels {0.738±0.01% lactic acid (LA) (T_1), 0.815±0.005% LA (T_2) and 0.927±0.01% LA (T_3)} of plain set yoghurt made employing ultrafiltration technique was investigated on physical, textural and sensory properties. Water holding capacity was observed to be significantly (p<0.05) higher in T_2 compared to T_1 and T_3. Textural attributes increased significantly (p<0.05) with increasing yoghurt acidity level. Treatment T_1 had significantly (p<0.05) lower flavour and acidity scores. Body & texture and overall acceptability scores were observed to be significantly (P<0.05) higher in T_2 treatment. Hence, maintaining yoghurt acidity of around 0.815±0.005% LA during incubation was observed to be optimum.

Key words: ultrafiltration, retentate, whey syneresis, water holding capacity, textural attributes

Introduction

Yoghurt is a popular fermented dairy product consumed all over the world. It is formed by slow fermentation of lactose to LA by thermophillic yoghurt starter bacteria namely *Streptococcus thermophilus* and *Lactobacillus delbrueckii* subsp. *bulgaricus* (Lucey, 2002). Horiuchi *et al.* (2009) reported that the global sales of yoghurt in year 2006 were approximately US$ 40 billion. According to a recent research conducted by Global Industry Analysts Inc., it was predicted that by year 2015, global yoghurt consumption will reach 20.6 million tons, equaling US$ 67 billion in sales. Asia presents a huge opportunity due to the rising incidence of lifestyle-related health concerns, such as diabetes and obesity, brought on by rapid economic development and rising income levels, (Anon, 2010).

The set yoghurt is produced by packaging the yoghurt mix into individual containers before fermentation. As a commercial product, it is important that the set yoghurt has curd with sufficient hardness to stand up to the impact caused by shaking during transportation (Horiuchi *et al.* 2009). Nielsen (1975) suggested that the texture of set yogurt should be firm enough to remove it from the container with a spoon. According to Lewis and Dale (1994), set yoghurt should have a glossy surface appearance without excessive whey. Whey Syneresis is a major defect of set-style yoghurt (Lucey 2001). The formulation of yoghurt products with optimum consistency and stability to whey syneresis is of primary concern to the dairy industry (Biliaderis *et al.* 1992). Factors influencing yoghurt texture and whey syneresis include total solids (TS) content especially proteins, homogenization, type of culture, acidity resulting from growth of bacterial cultures and heat treatment of milk (Harwalkar and Kalab 1986).

Acidity of yoghurt is a consequence of lactic acidification obtained at the end of the incubation period and post acidification during the storage of yoghurt (Beal *et al.*, 1999). According to the pevailing

standards of yoghurt, final acidity vary between 0.7% (IDF,1992) to 0.9% LA (FDA,1996). FSSA (2006), India requrement is to have 0.85% to 1.2% LA during the shelf life of yoghurt. Acidity influences the quality attributes of yoghurt such as flavour, textture, whey syneresis, shelf life etc. Therefore, an attempt was made to improve quality of yoghurt made employing ultrafiltration (UF) technique by stopping incubation at various titratable acidity (TA) levels and made recommendations thereof.

Materials and Methods

Materials

Raw cow skim milk and cream (about 50-55% fat) was obtained from Experimental Dairy of National Dairy Research Institute, Karnal. Well reputed brand (Nestle') of commercial yoghurt containing *Streptococcus thermophilus and Lactobacillus delbrueckii subsp. bulgaricus* was used as the starter culture for the production of yoghurt.

Methods

Ultrafiltration of cow skim milk and production of experimental yoghurts

Cow skim milk was heated to 80°C, cooled to 55-60°C and transferred to the balance tank of pilot UF plant {Tech-Sep, France with tubular module (channel diameter, 6 mm) having ZrO_2 membrane (membrane surface area, 1.68 m^2 and membrane molecular weight cut off, 50,000 Dalton)} and ultrafiltered at 50-55°C to 5 fold UF concentration. Cow skim milk was standardized to 13.9% TS and 3.3% fat by adding calculated amount of 5 fold UF cow skim milk retentate and cow milk cream, respectively. Resultant standardized milk was pre-heated to 65-70°C; homogenized in a two-stage homogenizer (M/s Goma Engineers, Mumbai) at 2000 and 500 psi at 1st and 2nd stages, respectively; heat treated at 85°C/30 min in a thermostatically controlled water bath (NAVYUG, India); cooled immediately in an ice water tub to 42-45°C; inoculated with 2% of yoghurt culture; mixed; filled in clean polystyrene cups; covered with lids and incubated at 42±1°C. Incubation was stopped at different TA levels viz. 0.738±0.01% LA (T_1), 0.815±0.005% LA (T_2) and 0.927±0.01% LA (T_3). Yoghurts were then immediately transferred to a refrigerator maintained at 4±1°C. Respective pH of the samples were observed to be 4.77±0.02, 4.58±0.01 and 4.51±0.02. Quality of yoghurt was evaluated in terms of sensory and physical parameters including textural attributes. Experiment was repeated 3 times.

Physicochemical analysis

A pH meter (PHAN LABINDIA Model, Labtek Eng. Pvt. Ltd. India) was used to determine pH of yoghurt during incubation. Titratable acidity was determined using procedure recommended in BIS (1981a). Fat content of skim milk and UF cow skim milk retentates were determined as per the method given in BIS (1981a), whereas, in cream as per the methods given in BIS (1977)

Spontaneous whey syneresis (SWS)

Siphon method described by Amatayakul *et al.* (2006) was used with slight modifications to determine the SWS. A cup of yogurt (100 ml) was tilted immediately after removing from the refrigerator at an angle of 45° to collect the surface whey. Collected whey was siphoned out with a graduated syringe with a needle. The siphoning was performed within 10 s to avoid forced leakage of whey from the curd. The value was taken directly as the percent SWS.

Water Holding Capacity (WHC)

The WHC was measured by a centrifuge method given by Supavititpatana *et al.* (2009). Within 12 h of the production of yogurt, a 10 g sample was centrifuged at 2,000 g for 60 min at 10±1°C. The supernatant was removed within less than 10 min and the wet weight of the pellet was recorded. The WHC was expressed as follows.

$$\text{WHC (\%)} = \frac{\text{Pellet (g)}}{\text{Sample (g)}} \text{x} 100$$

Textural attributes

Texture analysis was carried out according to the method given by Kumar and Mishra (2003) with slight modifications, using a TA-XT2i Texture analyser (M/s Stable Micro Systems, UK) fitted with a 25 kg load cell and was calibrated with a 5 kg standard dead weight prior to use. For determining the textural attributes,

the pasteurized and cooled standardized milk was filled up to 80 ml in 100 ml clean glass beaker and incubation was carried out. Experiments were carried out by compression tests that generated plot of force (N) versus time (s). A 25 mm perplex cylindrical probe was used to measure texture of yoghurt samples at a temperature of 10±0.5°C performing four repetitions. During analysis the samples were compressed up to 20 mm of their original depth. The speed of the probe was 0.5 mm/s during the compression, 2 mm/s during pre-test and relaxation. From the resulting force-time curves, firmness, stickiness, work of shear (WoS) and work of adhesion (WoA) were calculated using the Texture Expert Exceed software (version 2.55) supplied by the manufacturer along with the instrument.

Sensory evaluation

On the basis of desirable attributes for good quality yoghurt, the 100 point score card suggested by Ranganadham and Gupta (1987) was used for the sensory evaluation of yoghurt. The values of 100 point score were divided for flavour, body & texture, acidity, colour & appearance and container and closure viz., 45, 30, 10, 10 and 5, respectively. Yoghurts were sensory evaluated at 10±1°C by a panel of 8 trained judges at National Dairy Research Institute, Karnal.

Statistical analysis

The results obtained in the present study were subjected to one-way analysis of variance (ANOVA) using SPSS Version 16. LSD was used for mean comparisons. Critical difference (CD) was calculated according to the method described by Rangaswamy (1995). Significant differences were determined at 95% level of confidence.

Results and Discussion

Effect of TA of yoghurt during incubation on whey syneresis and WHC

Whey syneresis was observed only in T_3 treatment (Tab 1). However, it was not significantly different between treatments. When yoghurts were kept in the incubator for more time (to develop acidity further), it was observed that whey syneresis started to appear. Water holding capacity was observed to be significantly (p<0.05) higher in T_2 compared to T_1 and T_3. Further, WHC was significantly (p<0.05) higher in T_3 compared to T_1. Water holding capacity was observed to be highest in T_2 followed by T_3 and T_1. Corresponding values were 64.68, 63.60 and 62.78%, respectively (Tab 1). Sodini *et al.* (2004) mentioned that the yoghurt pH had a significant effect on WHC. There is a relationship between TA and pH and it affects WHC. According to the current study, low acidity/high pH (T_1) and high acidity/low pH (T_3) treatments had significantly (p<0.05) low WHC than the moderate treatment (T_2).

According to Aguilera and Kessler (1989) curds with high pH had a poor WHC in GDL-acidified gels. Harwalkar and Kalab (1986) noticed that, within the range of common final pH encountered for yoghurt manufacture, reduction in the pH, slightly decreased the WHC of the yoghurt. They reported WHC of 67% and 65% for yoghurt having pH 4.50 and 3.85, respectively. Findings of the current study also agreed with earlier reports.

Table 1:
Physical and textural parameters* of plain yoghurt as affected by TA during incubation

TA level (% LA)	Whey syneresis (%)	WHC (%)	Firmness (N)	Stickiness (N)	WoS (N.s.)	WoA (N.s.)
0.738± 0.01	0	62.78[a]	1.61[a]	-0.33[a]	52.27[a]	-1.94[a]
0.815± 0.005	0	64.68[c]	1.92[b]	-0.43[b]	54.62[b]	-2.34[b]
0.927± 0.01	0.08	63.60[b]	2.10[c]	-0.50[c]	57.43[c]	-2.78[c]
$CD_{0.05}$	**NS**	**0.66**	**0.09**	**0.02**	**0.98**	**0.28**

[a,b,c]**Means with different superscripts within each column differ significantly (p<0.05)**

Effect of titratable acidity of yoghurt during incubation on textural attributes

Firmness, stickiness, WoS and WoA increased significantly (p<0.05) with increasing yoghurt acidity level (Tab 1). Rönnegard and Dejkmek (1993) studied the linear viscoelastic properties of yoghurt fermented

to different pH values. They observed a higher complex viscosity and a lower angle shift when the pH was decreased from 4.50 to 4.25. Beal *et al.* (1999) showed that there was a significant effect of final pH on viscosity of yoghurt, and with decreasing pH, viscosity was reported to be increased. Harwalkar and Kalab (1986) reported an increase of 20% of gel firmness when the final pH was decreased from 4.50 to 3.85.

Effect of TA of yoghurt during incubation on sensory attributes

Effect of TA during incubation on sensory attributes of plain yoghurt is presented in Tab 2. It was observed that all the sensory scores significantly (p<0.05) different between treatments (Tab 2). Flavour score was highest (40.63 out of maximum possible 45) in T_2 and it was not different from the flavour score obtained by T_3. Treatment T_1, which was having lowest acidity level during incubation had lowest flavour score (38.94 out of maximum possible 45) and it was significantly (p<0.05) lower than T_2 and T_3 treatments. One of the flavour compounds that impart disctinctive flavour to yoghurt is lactic acid (Beshkova *et al.*, 1998; Chaves *et al.*, 2002) among others. Yoghurts were served to sensory panel nearly after 24 hours of storage. When the TA of yoghurt is low during incubation (T_1), TA at the time of consumption is also less. Lactic acid production may be insufficient to give a distintive flavour caracteristic to final product and this may be the reason to have significantly (p<0.05) lower flavour scores of the yoghurts of T_1 treatment compared to other tratments. On the other hand, T_3 treatment had lower flavour score than the treatment T_2 indicating that the higher acidity is also not favourable. Hence, it can be concluded that, treatment T_2 having 0.815±0.005% LA/4.58±0.01 acidity/pH value during incubation of yoghurt, is the best among tested treatments. This product had acidity/pH level of 0.860±0.005% LA/4.56±0.01 after 24 h of refrigeration, which agrees with the current FSSA (2006) regulations of India for final TA of yoghurt.

Table 2:
Sensory scores* of plain yoghurt as affected by TA during incubation

TA level (% LA)	Flavour	Body & texture	Acidity	Colour & appearance	Overall acceptability
0.738± 0.01	38.94[b]	26.19[b]	7.13[b]	8.59[a]	85.84[c]
0.815± 0.005	40.63[a]	27.22[a]	8.13[a]	8.25[a]	89.22[a]
0.927± 0.01	40.31[a]	26.25[b]	7.88[a]	7.81[b]	87.25[b]
$CD_{0.05}$	0.939	0.560	0.577	0.491	1.589

[a,b,c]**Means with different superscripts within each column differ significantly (p<0.05)**

Yoghurt acidity score also followed a similar trend as flavour scores between treatments indicating that acidity is a distinctive characteristic of flavour of yoghurt. In manufacturing yoghurt, fermentation is stopped at a pH inferior to 4.6. It could vary, depending on the process conditions from 4.6 to 4. It has a significant effect on sensory properties such as acidity, flavour, and texture (Lucey and Singh, 1998; Sodini, *et al.*, 2004).

Body and texture score was observed to be significantly (P<0.05) higher in T_2 treatment followed by T_3 and T_1. When acidity was less and pH was high (T_1), the curd was loose and obtained lower body and texture score. The acidification process results in the formation of three-dimensional network consisting of clusters and chains of caseins (Mulvihill and Grufferty, 1995). This completes at pH 4.6 which was the IEP of casein. Hence, pH above 4.6 is not favourable to have yoghurt having a good body and texture and current study further confirmed it. On the other hand, T_3 treatment which had highest TA and lowest pH combination, also obtained significantly (p<0.05) lower body and texture score than T_2 treatment. With the increase of TA, whey syneresis was noted on the top of the curd and the yoghurt body was observed to be little shrunk. This might be the reason to have lower score for body and texture of yoghurts in treatment T_3. This

shrinkage is due to the rearrangement of the three dimensional protein network of the yoghurt. Martin *et al.* (1999) reported that, stirred yoghurt obtained at a pH between 4.4-4.2 is more thick-in-mouth and consistent than those obtained at a pH between 4.8-4.7. However, in the current study, the pH of the best treatment that obtained highest body & texture score was 4.58±0.01 which was higher than the value reported by Martin *et al.* (1999). Colour and appearance score was significantly (p<0.05) higher in yoghurt made from T_1 compared to T_3 treatment. T_1 had the highest score of 8.59 out of maximum possible 10 followed by 8.25 in T_2. T_3 treatment obtained lowest score for colour and appearance (7.81) and this is due to the appearance of whey on the surface of the yoghurt. Overall acceptability score reflected the scores obtained by all sensory parameters and it was significantly (p<0.05) higher in T_2 compared to T_1 and T_3. Hence, maintaining yoghurt acidity of around 0.815±0.005% LA during incubation was observed to be optimum.

Correlations between some important parameters

Pearson's correlation coefficients were determined for selected parameters to check whether there is any correlation and to determine the strength of the correlation. It was observed that the acidity/pH level during incubation of yoghurt had a significant (p<0.05) positive correlation with firmness of the yoghurt (r=0.958). Further, it had a significant (p<0.05) positive correlation with flavour score of yoghurts. This indicates that with increasing acidity/pH level, the flavour score was also increased in tested acidity/pH levels. However, it is important to note that the highest flavour score was obtained by T_2 treatment even though, it was not different compared to the flavour score obtained by T_3 treatment. Other sensory attributes such as body & texture, acidty and overall acceptability scores were not significantly correlated with acidity/pH levels. Apart from that, acidity score was significantly (p<0.05) correlated with flavour score (r=0.821) and overall acceptability score (r=0.825). Further, flavour score was significantly (p<0.05) correlated with overall acceptability score (r=0.896).

Conclusion

Physical, textural and sensory quality of plain set yoghurt made employing UF technique could be improved by stopping incubation at 0.815±0.005% LA. The optimum quality yoghurt had 64.68% WHC, 1.924 N firmness, -0.434 N stickiness, 54.616 N.s. WoS and -2.339 N.s. WoA with no whey syneresis. Further increase of acidity has adverse effect on quality of the product and hence, stopping incubation at 0.815±0.005% LA would be recommended for the production of good quality plain set yoghurt.

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References

Aguilera, J. M. and Kessler, H. G. (1989). Properties of mixed and filled type dairy gels. *J. Food Sci.*, **54**: 1213–1217.

Amatayakul T., Sherkat F. and Shah N. P. (2006). Physical characteristics of set yoghurt made with altered casein to whey protein ratios and EPS-producing starter cultures at 9 and 14 % total solids. *Food Hydrocoll.,* **20**: 314-324.

Anon (2010). Yoghurt sales may reach US $ 67 billion by 2015. [Last accessed: 5-6-2013]

Beal, C., Skokanova, J., Latrille, E., Martin, N. and Corrieu, G. (1999). Combined effects of culture conditions and storage time on acidification and viscosity of stirred yoghurt. *J. Dairy Sci.*, **82**: 673-681.

Beshkova, D. Simova, E. Frengova, G and Simov, Z. (1998). Production of flavour compounds by yoghurt starter cultures. *J. Ind. Microbiol. & Biotech.,* **20**: 180–186.

Biliaderis, C. G., Khan, M. M. and Blank, G. (1992). Rheological and sensory properties of yoghurt from skim milk and ultrafiltered retentate. *Int. Dairy J.*, **2**: 311-323.

BIS (1981a). Rapid examination of milk. In: *Handbook of Food Analysis (SP: 18 - Part XI): Dairy Products*. Bureau of Indian Standards. Manak Bhavan, New Delhi. pp: 5-20.

BIS (1977). Determination of fat by ther Gerber method - Milk products. IS: 1224 (part II) - 1977. Bureau of Indian Standards. Manak Bhavan, New Delhi.

Chaves, A. C. S. D., Fernandez, M., Lerayer, A. L. S., Mierau I., Kleerebezem, M. and Hugenholtz, J. (2002). Metabolic engineering of acetaldehyde production by *Streptococcus thermophilus. Appl. Environ. Microbiol.*, **68** (11): 5656–5662.

FDA. (1996). Yoghurt. 21 CFR 131.200, Code of Federal Regulations. U.S. Dept. of Health and Human Services, Washington, DC.

FSSA (2006). Food Safety and Standards Act. Food Safety and Standards Authority of India, New Delhi.

Harwalkar, V. R., and Kalab, M. (1986). Relationship between microstructure and susceptibility to syneresis in yoghurt made from reconstituted nonfat dry milk. *Food Microstruct.,* **5**: 287-294.

Horiuchi, H., Inoue, N., Liu, E., Fukui, M., Sasaki, Y. and Sasaki, T. (2009). A method for manufacturing superior set yoghurt under reduced oxygen conditions *J. Dairy Sci*., **92**: 4112–4121.

IDF (1992). General Standards of Identity for Fermented Milks. Standard 163. International Dairy Federation, Brussels.

Kumar, P. and Mishra, H. N. (2003). Effect of mango pulp and soy milk fortification on the texture profile of set yoghurt made from buffalo milk. *J. Text. Stud.,* **34**: 249-269.

Lewis, M. and Dale, R. H. (1994). Chilled yoghurt and other dairy desserts. In: *Shelf life evaluation of foods.* (Eds. C. M. D. Man and A. A. Jones). Blackie Academic and Professional, UK. pp: 127-155.

Lucey, J. A. (2001). The relationship between rheological parameters and whey separation in milk gels. *Food Hydrocoll.*, **15**: 603–608.

Lucey, J. A. (2002). Formation and physical properties of milk protein gels. *J. Dairy Sci*., **85**: 281–294.

Lucey, J. A. and Singh, H. (1998). Formation and physical properties of acid milk gels: A review. *Food Res. Int.*, **30**: 529-542.

Martin, N. C., Skokanova, J., Latrille, E., Beal, C. and Corrieu, G. (1999). Influence of fermentation and storage conditions on the sensory properties of plain low fat stirred yoghurts. *J. Sens. Stud.*, **14**: 139–160.

Mulvihill, D. M. and Grufferty, M. B. (1995). Effect of thermal processing on the coagulability of milk by acid. In: *Heat induced Changes in Milk. Special Issue No. 9501* (Ed. P. F. Fox). International Dairy Federation, Brussels. pp: 188-205.

Nielsen, V. H. (1975). Factors which control the body and texture of commercial yoghurts. *Am. Dairy Rev*., **37**: 36–38.

Ranganadham, M. and Gupta, S. K. (1987). Sensory evaluation of *dahi* and *yoghurt*. *Indian Dairyman*, **39** (10): 493-497.

Rangaswamy, R. (1995). A textbook of agricultural statistics. New Delhi: New Age

Ronnegard, E. and Dejmek, P. (1993). Development and breakdown of structure in yoghurt studied by oscillatory rheological measurements. *Lait.*, **73**: 371-379

Sodini, I., Remeuf, F., Haddad, S. and Corrieu, G. (2004). The relative effect of milk base, starter and process on yoghurt texture: A review. *Crit. Rev. Food Sci.,* **44**: 113-137.

Supavititpatana, P., Wirjantoro, T. I. and Raviyan, P. (2009). Effect of sodium caseinate and whey protein isolate fortification on the physical properties and microstructure of corn milk yoghurt. *CMU. J. Nat. Sci*., **8**(2): 247-263.

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Studies on tissue and cell cultures for plant regeneration of Purple coneflower (*Echinacea purpurea* L.)

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Abstract: Explants of leaf, root and petiole were taken from *in vitro* grown Purple coneflower (*Echinacea purpurea* L.) plants and their regeneration ability was compared by culturing these explants on media with various supplements. The regeneration ability in root and petiole explants was higher than that in leaf ones, and a combination of 0.3 mg/l benzyladenine (BA) with 0.01 mg/l naphthaleneacetic acid (NAA) in Murashige and Skoog (MS) basal medium was the most effective, yielding 100% shoot regeneration frequency and associated with the highest number of 1.84 shoots per explant. Explants with higher chromosome level need higher cytokinine concentration to achieve higher regeneration frequency. In the experiments, haploid and diploid plants showed higher regeneration potential at 0.3 mg/l BA whereastetraploid (2n=44) required a higher BA concentration of 0.5 mg/l to accelerate the ability. All explants from two and a half months old plantlets produced buds in high frequency; 1143.9 buds were regenerated from 1g explants. With the established regeneration culture system, it was possible to obtain a large number (1124864) of buds within one year period from one mother plant. For initiation of roots from the regenerated shoots and stimulation of growth of the plantlets, all shoots from different explants responded equally well on medium with 0.01 mg/l NAA.

Keywords: *Echinacea purpurea, in vitro,* regeneration, haploid, diploid plants, tetraploid plants

Introduction

Purple coneflower (*Echinacea purpurea* L; Compositae or sunflower family) is one of the most popular medicinal herbs. Recently, because epidemic diseases caused by viruses have become much more threatening, global demand for products of purple coneflower has been increasing.

The main prerequisite for the development of high-quality medicinal products is a consistent source of high-quality plant material (Murch *et al*. 2004). However, purple coneflower is heterozygous, the content of medicinal compounds might differ significantly among individual plants and the quality of the medicine manufactured from these plants might be not stable. Because of this, techniques for *in vitro* propagation of seedlings of elite genotype in purple coneflower have high application value. Plant regeneration in coneflower has been reported by culturing leaf and petiole ex-plants (Koroch *et al*. 2001; Roger *et al*. 2004; Choffe *et al*. 2000a; Kristen *et al*. 2000).

Plant regeneration in coneflower has been reported by culturing leaf and petiole explants.In the present paper, we report an efficient *in vitro* propagation culture protocol for this important medicinal plant.

Objectives

1). Establish simple and efficient *in vitro* culture system to regenerate buds and induction of roots which can be applied for agricultural practice such as propagation of elite clone.

2). To produce large number of uniform cells or cell clusters that is the most suitable material for re-differentiation studies.

Materials and methods

Investigation of the regeneration ability of explants from different maturity plantlets

Leaf, petiole and root explants of aseptic plantlets were cultured on MS basal medium supplemented with 0.3mg/l BA and 0.01 mg/l NAA to investigate the regeneration ability with the age of downer plantlets. The optimum plantlet age for shoot initiation was determined by comparing the regeneration ability of roots, petioles and leaves taken from plantlets of one and a half months, 2 months, 2 and a half months, and 3 months old.

Estimation of the capacity of plantlet production with the established methods

Five healthy plantlets were randomly selected as explant source and 50% of each type (root, petiole and leaf) of explants taken from these plantlets were cultured on regeneration medium. Regenerated buds were rooted and the resulted plantlets were again divided into explants of different kinds and cultured for regeneration of buds. This cycle was repeated again and again and all the healthy buds and plantlets produced from all explants were counted.

Rooting of adventitious buds

Healthy shoots longer than 1.5 cm regenerated from all explants types were isolated and inoculated on MS basal medium containing 0.01 mg/l NAA.

Data collection and analysis

All experiments were repeated at least once with a minimum of four replicates. Analysis of variance was carried out with the use of Statistical Analysis Systems (SAS version 9.2) software and DMRT tests were applied to compare the treatment means.

Results

Explants of leaf, petiole and root were inoculated on MS basal medium with BA at various combinations (0.1, 0.3, 0.9, 2.7 mg/l) and NAA at 0.01 mg/l. Most explants formed callus at the cut surface in two weeks, and the callus began to produce bud primodia in another one week. The primodia developed into adventitious buds afterwards. It was found that medium supplemented with 0.3 mg/l BA yielded the best results, allowing all the root and petiole explants and a higher percentage of leaf explants to regenerate adventitious buds (Table 1). It displayed 100% shoot regeneration form root and petiole explants associated with a high number of shoots per explants (1.84) without showing vitrification. A lower or higher concentration of BA was less effective; especially when higher concentration of BA used, not only the frequency of regeneration decreased, the quality of the regenerated buds also dropped as the symptoms of vitrification on the buds became evident. The callus observed with higher BA and NAA concentrations were brown and excessive necrosis, indicating toxic effects. Although difference in regeneration ability was observed among the three kinds of explants, quality of the regenerated buds from all the explants were alike. In most of the cases, regeneration ability in root and petiole explants was higher than that in leaf ones. Visual observations of the cultures revealed distinct routes of morphogenesis resulting in the formation of shoots in response to BA. According to these, the balance of auxin and cytokinine is a determining morphogenic factor of organogenesis.

On the bases of the above experiments, BA was used at 0.3 mg/l and NAA was tested at various concentrations (0.0, 0.01, 0.05, 0.15, 0.75 mg/l). Results of the experiments are summarized in Table.2. It is clear that concentration of NAA also played a very important role in regulating shoot regeneration. Explants of root and petiole were found to possess higher bud regeneration potential in all the NAA concentrations tested, and under the most suitable NAA concentration of 0.01 mg/l, explants of root and petiole had at least 30% higher regeneration potential than those of leaf.

Differences in maturity of plantlets greatly influenced the initiation of shoot buds (Table 3).

Percentage of shoot initiation from all root, petiole and leaf explants increased with increasing plantlet maturity upto two and a half months and after that negative effect was observed. Minimum regeneration potential (548.8 buds per 1 g of explants) was observed from one and a half months old plantlets whereas reached to maximum number (1143.9 buds per 1 g of explants) in two and a half months plantlets with showing less vitrification. The moderate regeneration ability was displayed from 3 months and 2 months old plantlets showing 745.7 and 782 buds per 1 g of explants respectively.

Discussion

Plant regeneration from petiole explants of *E. purpurea* was achieved by using only a small amount of BA (Choffe*et al*., 2000a), whereas, in the present study, BA (0.3 mg/l) in combination with NAA (0.01 mg/l) was the most effective in inducing adventitious shoot regeneration from all explants. Different explants are known to produce different types and frequencies of regenerative responses (Annadana*et al*., 2000); with hypocotyls being more responsive than other explants in many species (Gubis*et al*., 2003; Chae*et al*., 2004). It is apparent that the source of explants significantly affects the regenerative response of *E. purpurea.*

Conclusion

Regeneration ability in root and petiole explants of *E. purpurea* was higher than that in leaf ones.

All explants from two and a half months old plantlets produced buds in high frequency. Throughthe established regeneration system, it was possible to obtain a large number of bud productions within one year period.

References

Annadana S,Radamaker W, Rammana M,*et al*. 2000. Response of stem explants to screening and explant source as a basis for methodical advancing regeneration protocols for *chrysanthemum*. Plant Cell Tissue Organ Cult 62: 47-55

Chae W B, Choi G W and Chung I S. 2004. Plant regeneration depending on explant on explant type in *Chrysathemumcoronarium* L. J Plant Biotech 6: 253-258

Choffe K L, Victor J M R, Murch S J, *et al*. 2000a. *In vitro* regeneration of *Echinacea purpurea* L.: Direct somatic embryogenesis and indirect shoot organogenesis in petiole culture. *In Vitro*Cellula DevelopBiol Plant 36(1): 30-36

Gubis J, Lajchova Z, Farago J, *et al*. 2003. Effect of genotype and explant type on shoot regeneration in tomato (*Lycopersiconesculentum* Mill.) *in vitro*. Czech J Genet Plant Breed 39: 9-14

Koroch A, Juliani H R, Kapteyn J, *et al*. 2001. *In vitro* regeneration of *Echinacea purpurea* from leaf explants. Plant Cell Tissue Organ Cult 69: 79-83

Kristen L, Choffe, Jerrin M R, *et al*. 2000. *In Vitro* regeneration of *Echinacea purpurea* L.: Direct somatic embryogenesis and indirect shoot organogenesis in petiole culture. *In Vitro* Cellula Develop Biol Plant 36: 30-36

Murch S J, Liu C Z, Romero R M, *et al*. 2004. *In vitro* culture and temporary immersion bioreactor production of *Crescentia cujete*. Plant Cell Tissuse Organ Cult 78(1): 63-68

Roger J, Sauve, Margaret T, *et al*. 2004. *In vitro* regeneration of the Tennessee coneflower (*Echinacea tennesseensis*). *In Vitro* Cellula Develop Biol plant 40: 325- 328

Table 1. Comparison of the ability of shoot regeneration of different explants on media containing 0.01mg/l and various concentration of 0.3 mg/l BA

Explant	BA concentration (mg/l)							
	0.1		0.3		0.9		2.7	
	Regeneration (%)	No. buds per explant	Regeneration (%)	No. buds per explant	Regeneration (%)	No. buds per explant	Regeneration (%)	No. buds per explant
Leaf	37.5 c*	0.28 c	75.0 b	1.05 b	12.5 b	0.13 b	22.5 b	0.10 c
Petiole	87.5 b	0.80 b	100.0 a	1.84 a	87.5 a	0.83 a	40.0 a	0.25 b
Root	100.0 a	1.13 a	100.0 a	1.75 a	87.5 a	1.00 a	40.0 a	0.40 a

*Means fallowed by the same lower case letters in each column are not significantly different at 5% level in Duncan's Multiple Range Test.

Table 2. Comparison of the ability of shoot regeneration of different explants on media with various concentration of NAA with 0.3 mg/l BA

Explant	NAA concentration (mg/l)									
	0.0		0.01		0.05		0.15		0.75	
	Regeneration (%)	No. buds per explant	Regeneration (%)	No. buds per explant	Regeneration (%)	No. buds per explant	Regeneration (%)	No. buds per explant	Regeneration (%)	No. buds per explant
Leaf	50.00a*	0.71 b	59.4 b	1.01 b	43.75a	0.63 b	20.83 b	0.21 b	8.33 c	0.08 b
Petiole	56.25 a	1.07 a	91.66 a	1.54 a	45.83a	1.13 a	40.00 a	0.83 a	29.16 b	0.46 a
Root	59.40 a	1.04 a	93.75 a	1.73 a	50.00 a	1.27 a	45.00 a	0.83 a	40.00 a	0.44 a

*Means fallowed by the same lower case letters in each column are not significantly different at 5% level in Duncan's Multiple Range Test.

Table 3. Effects of plantlet age on regeneration of buds

Age of plant (months)	No of buds from 1 g of leaf explants	No of buds from 1g of petiole explants	No of buds from 1g of root explants	No of vitrified buds	Time taken to regenerate (days)
1.5	74.6 c*	235.4 d	236.8 c	38.8 a	24.4 c
2.0	102.0 b	352.0 b	328.8 b	24.2 b	24.6 c
2.5	194.5 a	443.6 a	505.8 a	11.6 c	28.2 b
3.0	94.6 b	319.9 c	331.2 b	13.0 c	30.6 a

*Means fallowed by the same lower case letters in each column are not significantly different at 5% level in Duncan's Multiple Range Test.

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Feeding preference of the predatory larvae of genus *Lutzia* (Diptera: Culicidae).

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Abstract: Mosquitoes are classified under the family Culicidae and comprise a monophyletic taxon belonging to order Diptera. Genus *Lutzia* belongs to subfamily Culicinae. Mosquitoes play a major role as vectors of many pathogens. The larva of *Lutzia* is known as predators of other mosquito larvae. To obtain some understanding of the predatory potential of *Lutzia*on *Chironomous, Aedes* and *culex*this quantitative study was undertaken. The consumption pattern of *Lutzia* isstatistically significance ($p<0.05$) with hours of interval and the consumption percentages statistically significance ($p<0.05$) with different species. *Lutzia* gave high preference for *Chironomous* larvae, *Aedes* and *Culex* respectively.

Keywords: Consumption, Feeding preference, Instar, *Lutzia*, Predatory larva

Introduction

Mosquitoes are classified under the family Culicidae and comprise a monophyletic taxon (Wood and Borkeat, 1989; Miller *et al.*, 1997) belonging to order Diptera. They are classified into three subfamilies which contain Anophelinae, Culicinae and Toxorhynchitinae (Goma, 1996; Scholt and Holm, 1985). About 3490 species are currently recognized (Harbach and Howard, 2007). Mosquitoes are found throughout the world except in places that are permanently frozen. Three quarters of all mosquito species live in the humid tropics and subtropics, where the warm moist climate is favorable for rapid development and adult survival and the diversity of habitats permitted the evolution of many species (Clements, 1992).

Culicids exhibit complete metamorphosis. The adult lays eggs on water surface. The juvenilepasses through both larval and pupal stages and larvae are anatomically different from adult and feed on different types of food. About 95% of species are restricted to fresh water (Grueber and Bradley, 1994) and feed generally on aquatic micro organisms such as bacteria, diatoms and algae and detritus. But some larvae from subfamily Toxorhynchitine and genus *Lutzia* are predatory and feed on invertebrates and other mosquito larvae(Rajasekharan and Chowdaiah, 1972). The growing mosquito larva moults four times forming a pupa which is non feeding stage after the third molt. Adult male and female normally feed on plant juice for their energy need, but Culicine and Anopheline female feed on blood for their requirement for protein for egg development (Mellanby, 1963; Scholtz and Holm, 1985). Toxorhynchitine female feed only on plant juices. The life span of adult mosquitoes ranges from a few days to several weeks but in temperate regions it is longer.

Mosquitoes are host for variety of pathogens and parasites including viruses, bacteria, protoctistans and nematodes. Many mosquitoes are vectors of pathogens that cause diseases in human and domestic animals. Fewer than 150 species largely confine to *Anopheles, Aedes* and *Culex* is indirect cause of morbidity and mortality among human and other organism (Zhang and Shear, 2007). Mosquitoes are vectors of several considerably dangerous diseases including Malaria, Dengue, Filarioses, Yellow fever and Encephalitis (Roberts, 1996). They also can be a nuisance and cause allergic reactions in people when they bite. Therefore mosquito control is essential.

Normally mosquitoes are controlled by three ways which are physical, chemical and biological control. Physically mosquitoes are controlled by locating and eliminating the breeding sites. The

environmental sanitation is a good method to control mosquitoes. Chemical control targets the adult and larvae. Adulticides and larvicides are used in control programmes. The chemical control of mosquitoes is not an environment friendly method. It affects the nontargeted living organisms and the environment adversely and also forms the resistant varities. Dichloro Diphenyl Trichloroethane (DDT) resistant mosquitoes have started to increase in numbers, especially in the tropics due to mutation and reducing the effectiveness of this chemical. These mutations can rapidly spread over vast areas if pesticides are applied indiscriminately (Chevillon *et al.*, 1999).

Hence biological control is important in the management practices of mosquitoes. Predators are potentially a possibility for biological control of mosquitoes. Control of mosquito larvae by various biologic means has been the subject of considerable research. Larvivorous fish such as *Gambusia affinis* (Myers, 1965) and *Poecilia reticulata* (Sasa *et al.*, 1965) are widely used in mosquito control. The pathogenic agents such as virus, bacteria, fungi and protozoa are under the study. *Bacillus thuringiensis* (Bt) is an insecticide with unusual properties that make it useful for pest control in certain situations. Bt is a naturally occurring bacterium common in soils throughout the world. Several strains can infect and kill insects. Because of this property, Bt has been developed for insect control. At present, Bt is the only "microbial insecticide" in widespread use. This is now used in mosquito control.

In New Orleans, Marten (1990) reported elimination of *Aedes albopictus* larvae from tire piles by introducing the copepod (*Macrocyclops albidus*). Other predators include dragonfly , which consume mosquito larvae in the breeding waters and adult , which eat adult mosquitoes.A few predacious mosquitoes are worthy of consideration at this stage. *Toxorhynchites* and *Lutzia* mosquitoes have obligatory predatory larvae but they have never been involved in disease transmission(Chow, 1972). However predators have specific ecological requirements and can only be used where their preferred living conditions are met. The life cycle of the predator is frequently not adapted to that of the target organism. So that it is unable on its own to bring about an effective reduction of the target population. Mass rearing and release of the predators or parasites is often expensive or impossible. This limits their large scale use in a number of specific habitats (Eilenberg and Hokkanen, 2006).). Mosquito larvae are mostly filter feeders but the larvae of genus *Lutzia* is known as predators of mosquito larvae for a long time (Rajasekharan and Chowdaiah, 1972).

Genus *Lutzia* belongs to subfamily Culicinae and it was earlier classified under sub genus *Culex Lutzia*. Presently it is classified as genus *Lutzia*. Sri Lanka has experience in dengue which is transmitted by vector mosquito. So this study mainly focuses on the use of mosquito genus *Lutzia* as a predator for other dipterans as biological control agentto over come the environmental hazards of chemical pesticides.

Materials and Methods

This study was conducted during the one year period from February 2009 to March 2010. The field study was conducted at the Eastern University premises at Vantharumoolai. The laboratory work was carried out at the special laboratory of Department of Zoology, Eastern University, Sri Lanka.

Preparation and maintenance of ovitraps

Plastic trays (29cm×24cm×6cm) with the capacity of 2500ml were used as artificial ovitraps. Two types of ovitraps were prepared. One was filled with straw soaked water and another one was filled with normal tap water. In these two ovitraps, water was poured more than ¾ volume of the tray and the water level was checked and maintained approximately in same level.

Fig.1:Photograph showing the two types of ovitraps

Sample collection

Sample collection was done from natural ponds and artificial ovitraps in the study area. Adult, egg raft, larvae and pupae were collected in the study area. Both types of ovitraps (Fig.1) were placed together in different localities under shadow place. These ovitraps were checked for *Lutzia* and prey larvae and sample was collected for laboratory study and the water was refilled for next round of collection.

Laboratory experiments

Fig.2: Photograph showing the larval rearing.

Fifteen plastic cups were filled with 70ml of filtered tap water. Then field collected healthy fifteen second/third instar of *Lutzia* larvae were placed in each cups individually and was starved for twenty four hours. Then each ten of same instar of genus *Culex* and *Aedes* larva and same size of *Chironomous* larva were provided as a prey for *Lutzia* larva. The cups were covered by mosquito net to prevent from other contamination of oviposition of flying organism. The total number of prey was thirty in each cup. Consumed prey larvae were counted every twenty four hours until all the predatory larvae were pupated and the surviving larvae of each three species were counted at morning time and eaten larva was replaced in each time to maintain the prey density as same. In this experiment twenty replicates were made.

Data analysis

Data were analyzed statistically using statistical package SAS 9.0 and Minitab 14.0. The data were subjected to one way analysis of variance (ANOVA) for prey preference and the differences among means were considered significant at a probability level of five percent ($P \leq 0.05$).

Results and Discussion

Two genera of mosquito such as *Culex* and *Aedes* and *Chironomous* were used in this experiment. In these three species *Culex* and *Aedes* are medically importance in disease transmission and*Chironomous* is pollution indicator. In this experiment filtered tab water was used to reduce the any food contamination with mosquito prey larvae.

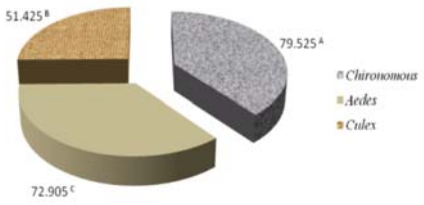
The results show the total percentage of the consumed number of each three of the prey larvae for consecutive of five days until the pupation of the predator *Lutzia* larvae and the average percentage of the each prey larvae for succeeding of five days in Table 1. *Lutzia* had the high preference for *Chironomous* larvae. One larva of *Lutzia* consumed 79.6% of *Chironomous*, 73.6% of *Aedes* and 51.2% of *Culex* from $2^{nd}/3^{rd}$ to pre pual stage in laboratory condition (Table 1). In each trial *Lutzia* shows same preference among three prey species.

Table 1: The total percentage of the consumed number of each three of the prey larvae until the pupation of the predator Lutzia larvae

		Percentage of prey larvae consumed by the predator from $2^{nd}/3^{rd}$ to pupation *		
Repeated No	Prey species	*Aedes*	*Culex*	*Chironomous*
1		73	51.2	78.5
2		69.1	49.9	77
3		75.2	51.7	81.1
4		72.8	48	78.8
5		78	55.1	82.5
	Total	368.1	255.9	397.9
	Average	73.6	51.2	79.6

* Average of 20 replicates in each trial.

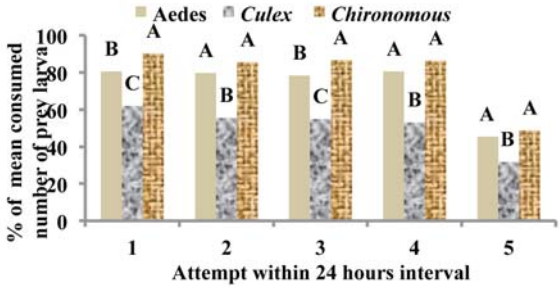
The results indicated that the total consumption percentage for the succeeding of five days until the pupation of predatory larva statistically significance ($P<0.05$) between three species of the prey which were used in this experiment. This will be clearly seen in the Fig.3. Total numbers consumed shows that the predator of *Lutzia* larva consumed higher percentage of *Chironomous* larvae between three species of treatment. Secondly *Lutzia* consumed *Aedes* in higher percentage than *Culex*. In this treatment consumption percentage of *Culex* species was very low compared to other two prey species.



A, B and C denote the statistical significance

Fig.3: Mean percentage of consumed number of different prey by the predator from the 2nd/3rd instar to pupation

In this experimental study, reading was taken at twenty four hours intervals until the pupation of the *Lutzia* larva. Graph in Fig.4 shows that the consumption pattern of *Lutzia* statistically significance ($p<0.05$) with hours of interval and the consumption percentages statistically significance ($p<0.05$) between the different treatment of preference. There is no statistically significance ($p>0.05$) between prey species and hours. That is in each and every hours consumption pattern of predator *Lutzia* did not change. Each 24 hours of reading *Lutzia* prefer *Chironomous* in higher percentage and then it prefers *Aedes* and finally prefers the *Culex* larvae in lower percentage compared to others. Analytical studies shows that in first and third twenty four hours there is a significant difference in consumption pattern between three prey species but there is no significant difference in consumption of *Chironomous* and *Aedes* in rest of the twenty four hours interval but there is a significance in consumption of *Culex* in that hours (Fig.4). During the last twenty four hours that is pre pupation time consumption pattern in three species of predator become very low.



*A, B and C denote the statistical significance

Fig. 4:Comparison of the mean % of consumed number of prey larvae by *Lutzia* to different prey until the pupation of predator

The colour of the *Chironomous* larvae effects in the predation by the predatory larvae of *Lutzia*. MacGregor (1924a) had reference to *Lutzia tigrips* eating *Chironomous* larvae. Haddow (1942) also observed that the *Lutzia* attack larvae and pupae of Chironomidae under natural conditions. Jin *et al*., (2004) also stated that the *Chironomous* larvae found in the gut content of 78.6% of *Lutzia fuscanus* larvae and mosquitoes remains in 2.5% of *Lutzia fuscanus* larvae.

If we concern about *Aedes* larvae apparently moved more frequently in the water than the others and this was confirmed by studying both the spontaneous movements and the movements induced after stimulation, of *Aedes* and *Culex sp., Aedes ganlbiue* and *Lutzia tigripes* larvae by Jackson, (1953). It was demonstrated from the experiment by Jackson, (1953) *Aedes aegypti* larvae were more active than any other groups of *Culex* and *Anopheles* larvae. In the case of *Aedes* and *Culex sp.* these two species may be particularly sensitive to some external stimuli such as the vibration caused by opening and closing the laboratory door or by the shadow of the observer walking past the basins. Due to these stimuli the duration of spontaneous movement of *Aedes* larvae was found to be significantly longer than that of the *Culex* larvae. Stimulated *Lutzia* larvae move spontaneously shorter periods than *Aedes* but longer than that of *Culex* species.Stimulated *Lutzia* will show more movement, but when no stimulation is given it will remain motionless more frequently than either *Culex sp*. It seems probable that this increase in amount of movement after stimulation can be accounted for the predaceous habit of the *Lutzia* larva. When a stationary *Lutzia* larva is approached or touched by its prey, it will continue to be active for several seconds afterwards (Jackson, 1953). So the *Lutzia* consumed lower number of *Culex* than *Chironomous* and *Aedes*. In this experiment the size of the prey species are approximately same in the each stage of them. So the size cannot effect in this experiment.

Conclusion

Among the three prey species such as *Culex* larva, *Aedes* larva and *Chironomous* larva, the consumption of *Lutzia* is statistically significance ($p<0.05$) with 24 hours interval and the consumption percentages are statistically significance ($p<0.05$) with the different

species. There is no significant ($p>0.05$) interaction effect between prey species and hours of interval. Larva of *Lutzia* prefer the *Chironomous* and *Aedes*higher than *Culex*.

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References

Clements, A. N. (1992). The biology of mosquitoes.Volume 1, Development, Nutrition and Reproduction, Chapman and Hall, London.ppXIII-XV.

Chevillon , C., Raymond, M., Guillemaud, T., Lenormand, T and Pasteur, N. (1999). .68 (1-2): 147–57.

Chow, C.Y. (1972). Alternative methods of mosquito control. *WPR.VBC*. 8:1-8

Eilenberg, J. and Hokkanen, H. M. T. (eds). (2006). An ecological and social approach to biological control. *Springer.*227-245.

Goma, L. K. H. (1996). The mosquito.Hutchinson and Co (publishers) ltd, London.pp 144.

Grueber, W. B. and Bradley, T. J. (1994). The evolution of increased salinity tolerance in larvae of Aedes mosquitoes: A phylogenetic analysis, *physiological Zoology* 67 (3): 566-579.

Harbach, R.E. and Howard, T.M. (2007). Index of currently recognized mosquito species (Diptera: Culicidae). *European Mosquito Bulletin*,23: 1–66.

Jackson, N. (1953). Observation on the feding habits of a predacious mosquito larvae, *Culex tigripes*. *Proc. Roy. Ent. Soc. Lond. A*, 28: 153-159.

Jin L., Luo J., Fu Y and Xu S. (2006). Prey and feeding behavior of larval *Culex* (*Lutzia) fuscanus* (Diptera: Culicidae) in Shantou, Guangdong Province, China. *Journal of medical entomology.* ISSN 0022-2585. 43: 785-786.

MacGrecor, M. E. (1924a). Report on the Anophelinae of Mauritius and on certain aspects of Malaria in the colony with recommendations for a new anti- malarial campaign. London: 1-48.

Marten, G .G. (1990b).Evaluation of cyclopoid copepods for *Aedes albopictus* control in tires.*J, am .Mosq . Control Assoc* .6 : 681- 688.

Mellanby, H. (1963). Animal life in fresh water .A guide to fresh water invertebrates.Methuen and Co ltd., London.pp 308.

Miller, B.R., Crabtree, M.B. and Savage, H.M. (1997). Phylogenetic relationships of the Culicomorpha inferred from 18S and 5.8S ribosomal DNA sequences Diptera: Nematocera). *Insect Molecular Biology*, 6:105–114.

Myers, G. S.(1965). Gambusia, the fish destroyer.*Trop. Fish Hobbyist (New Jersey)*. January issue : 31-32 and 53-54

Rajasekharan, P. T. and Chowdaiah, B. N. (1972).Biological markers in feeding experiments of mosquito larvae (*Culex (Lutzia) raptor*).*Experientia* 28: 981

Roberts, L. S. and Janovy, J: (Jr).(1996). Foundations of parasitology.Fifth edition.Wm. C. Brown publishers, Dubugue. 659pp.

Sasa, M. *et al*. (1965). Studies on mosquitoes and their natural enemies in Bangkok. Part 3. Observation on a mosquito eating " guppy". *Jap. J. exp. Med.*35: 63-80

Scholt, C. H. and Holm, E. (1985). Insects of southern Africa.Butterworths, Durban, South Africa, pp 502.

Wood, D.M and Borkent, A. (1989). Phylogeny and classification of the Nematocera, pp. 1333–1370. *In*: McAlpine, J.F. & Wood, D.M. (Eds), *Manual of Nearctic Diptera*. Vol. 3. Research Branch Agriculture

Canada Monograph No. 2, Canadian Government Publishing Centre, Hull, Quebec.

Zhang, Z. Q. and Shear, W.A. (Eds) (2007). Linnaeus Tercentenary: Progress in Invertebrate Taxonomy. *Zootaxa*, 1668 :1–766.

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Water footprint of chicken egg production under medium scale farming conditions of Sri Lanka: An analysis

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Abstract: Water footprint (WF) reflects how efficiently water has been utilized in the production cycle of a particular product or service. Under the production conditions of the farm of the Faculty of Agriculture, University of Ruhuna, Sri Lanka studied, the WF of Chicken Egg was calculated as 3734 m^3/ton. Other than drinking and servicing water, feed water accounted over 99% of the WF of egg production. It is concluded that through appropriate interventions, both at policy and industry level, water footprint of egg production systems can be lowered substantially.

Keywords: Water footprint, Layers, Eggs, Feed

Introduction

Water insufficiency and insecurity are among the greatest challenges caused by the climate change and global warming. Freshwater resources could be strongly affected by climate change. Increasing water scarcity and insecurity will lead to more deaths from drought and water-borne disease, political conflict over limited resources, and loss of freshwater species (Arnell, 1999) and phenomena's which associated with climate change such as more heat-waves over land areas, more frequent or intense floods, melting glaciers, higher water temperatures, increased rainfall variability are predicted to decrease equal distribution of water.

Water footprint of a product is a key criterion that reflects the water efficiency of its production. An analysis of the WF can also be used to identify the suitable strategies that can be adopted to produce a particular product at lowest WF. The objectives of this paper are 1) to determine the WF of chicken egg production under medium-scale farming conditions and production parameters of Sri Lanka and, 2) to explore the possible strategies to reduce the WF of chicken egg production. Chapagain and Hoekstra (2003) have defined the virtual-water content of a product (a commodity, good or service) as "the volume of freshwater used to produce the product, measured at the place where the product was actually produced".It refers to the sum of the water use in the various steps of the production chain. The main components of the WF were feed, drinking and servicing water. Ecological costs such CO_2emission and uses of high amount of water of livestock production systems are higher than those of crops. Some argue that consumption of livestock products should be minimized as they are hiving higher ecological cost indicators such as high WFs. However, it has been predicted that global animal product consumption including that of poultry will increase sharply in next few decades. Therefore, the means of reducing WF of animal products are of importance. WF values of range of agricultural products of different countries have been reported by Chapagain and Hoekstra, (2003) and Hoekstra and Hung, (2002). However, above calculations are based on a number of general assumptions which do not represent the actual farming conditions and production parameters. A careful analysis on how the WF value of a particular product has been computed can be used to identify appropriate strategies to reduce the WF values. Use of agricultural by-products such as rice bran, coconut poonac at higher levels was also identified as an important strategy. Policy level interventions are required to encourage ration formulators to consider water footprint values, in addition to nutrient compositions and prices of the feed in the ration formulation process.

Materials and Methods

As far as possible, the actual production conditions and parameters of the poultry unit of the Farm of the Faculty of Agriculture, University of Ruhuna were used as a model for the analysis. The methodology used by Mekonnen and Hoekstra, 2010; Champagain and Hoekstra, 2003, was used with relevant modifications. Assuming that the average egg production of a layer is 270 per year, an egg weighs about 53.75 grams, it was calculated that 73 layers are required to produce 1 Ton of eggs for an year (Table 1).

Table 1: Calculation of eggs per ton

Average egg weight per period	53.75 Grams
Eggs per ton	18604.65
Eggs per layer per year	270
Layers need to produce 1 ton of eggs	68.9037
No of layers, assuming 5% mortality	72.45

The main components of the WF were feed, drinking and servicing water. During the production cycle, six on-farm mixed rations were fed. The ingredient compositions of the rations and the main steps of WF calculation and are given in Table 2. Water Footprint values and the product fractions (PF) of the feed ingredients were collected from data bases (Mekonnen and Hoekstra, 2010). Based on the average feed intakes of layers at different stages of growth basis (0 to 4^{th}, 4^{th} to 10^{th}, 10^{th} to 17^{th} , 17^{th} to 22^{nd} , 22^{nd} to 28^{th} and 28^{th} to 52^{nd} weeks), the feed consumptions at respective periods were determined. The feed water component was the sum of water involved in the production and processing of feed ingredients and water required for feed preparation and mixing. To determine the water contribution of the feed ingredients, the WFs of each feed ingredient in the rations was multiplied by the amount of the respective ingredient, consumed. Assuming the drinking water intake of layers are as 2.5 times as total feed intake, the drinking water requirement was calculated. The servicing water component was assumed to be 50% of drinking requirement (Table 2).

Table 2. Ingredient compositions of the rations fed at different stages and the main steps of the water footprint calculation

Ingredient	Ingredient (%) for weeks of age									
	0-4	5-10	11-17	18-22	23-28	29-52	WF	PF	WF*PF*	(m^3) *
Maize meal	25	10	12	12	10	10	3203	1	3203	32.87
Rice Polish	30	38	40	40	40	40	3168	0.1	316.8	3.90
Broken rice	12	15	15	11	11	10	2497	0.15	374.55	1.84
Coconut oil meal	0	7	15	11	2	6	834	1	834	0
Gingerly oil meal	6.5	9	4	4	9	4	2847	1	2847	7.59
Soya oil meal	18	13	5	11	12	17	4851	0.85	4123.35	30.47
Fish (meal (Danish)	6	5	0	3	0	1.5	7130.97	0.85	6061.325	14.93
Fish meal (local)	0	0	5	2	5	0	7130.97	0.85	6061.325	0
Coconut Oil	0	0	0	0	0	0	4490	1	4490	0
Hypromeal	0	0	0	0	0	0			0	0
Meat and bone meal[1]	1	1.5	2	0	2	1	8974.35	0.85	7628.198	3.13
									Total	94.75
							Total			
Feed intake (kg)	41.055	138.62	237.1	269.51	327.4	1363	2377.32 kg			
Feed water (m^3)	94.75	244.66	348.1	408.18	560.49	2067	3724.10 m^3			
Total feed water										3724.10 m^3
Feed preparation[2]										1.18 m^3
Servicing water[3]										2.97 m^3
Drinking water[4]										5.94 m^3
Water foot print (m^3/ton)										3734.197m^3

* This column gives calculation only for the period of 0-4 weeks

1. Crude protein content of meat and bone meal is 1.85 times higher than soybean meal. Since WF of fish meal is not available, WF of meat and bone meal was assumed to be 1.85 times that of soybean meal.
2. 50% of the feed consumed (Chapagain and Hoekstra, 2003)
3. 50% of the drinking water (Chapagain and Hoekstra, 2003)
4. 2.5 x feed intake (Nayanarasi and Atapattu, 2008)

WF: Water Foot Print

PF: Product Fraction

Results and Discussion

The WF of the layer egg production under the current production conditions and parameters of the poultry unit of the Farm of the Faculty of Agriculture, University of Ruhuna was calculated to be 3734.19 m^3 / ton. However, the WF value caculated was much lower than value reported by Chapagain and Hoekstra (2003); 9070 m^3/ton for the egg production in Sri Lanka. Having rice bran, broken rice, maize, soybean meal and coconut oil meal as major feed ingredients the ration used can reasonably represent a common Sri Lankan layer diet. The other production parameters were also more or less similar to typical Sri Lankan

conditions. In contrast, the study of Chapagain and Hoekstra (2003) was based on a number of general assumptions. Importantly, assumption that Sri Lanka adopts a mixed system of poultry management is far from reality. Their calculations were based on a number of generalizations assuming that the farming system is a mixed one. Even though the production parameters were lower than commercial industry systems of layer chicken management, the production system of the farm studied can best be classified as an industrial system. The difference in the values reported in this study and the one reported by Chapagain and Hoekstra (2003) may mainly be due to those reasons.

The contribution of drinking and servicing water for the total WF were negligible (0.15 and 0.07% respectively). Feed water accounted over 99% of the WF of egg production and was identified as the most feasible aspect for the manipulation to reduce the WF. The contribution of each feed ingredient to the total feed water is shown in Table 3.

Ingredient	Mean % in six rations	% Contribution of the feed water
Maize meal	13.16	21.84
Rice Polish	38	8.03
Broken rice	12.33	2.64
Coconut oil meal	6.83	3.65
Gingerly oil meal	6.08	9.12
Soya oil meal	12.66	37.42
Fish (denis)	2.58	6.17
Fish meal (local)	2	5.47
Meat and bone meal	1.25	5.61

Modern layers could produce up to 360 eggs per year and thus there was a clear gap between the actual farm level feed conversion efficiency and the potential. Therefore, improvements in the management conditions towards the exploiting full genetic potentials of the birds are of importance to reduce the WF. Soya bean meal and maize meal were the highest contributors to the feed water (37 and 21%). Water efficient production systems for these crops are important to reduce the amount of total feed water.

Use agricultural by-products such as rice bran, coconut poonac at higher levels is also suggested as a strategy of lowering WF. This is mainly due to the lower product fractions of those ingredients. However, use of such materials is limited due to poor performance. Suitable strategies, such as the use of exogenous enzymes should be developed to mitigate the adverse effects of associated with higher inclusion levels of agricultural by-products on production efficiency

Policy level involvements may be needed in future so that ration formulators are required to consider water footprint values of the feeds, in addition to nutrient compositions and prices of the feed ingredients in the ration formulation process.

It is concluded that water footprint of chicken egg production under medium scale farming conditions of Sri Lanka is 3743m^3/ton. Through suitable interventions, both at policy and industry level, water footprint of egg production systems can be lowered significantly.

References

Arnell, N. W.(1999) Climate change and global water resources. Global Environmental Change 9, s31

Atapattu, N.S.B.M. (2011).Water footprint of broiler meat production in Sri Lanka: An analysis Proceedings of 16th International Forestry and Environment Symposium 2011 University of Sri Jayewardenepura, Nugegoda, Sri Lanka.

Chapagain, A.K. and Hoekstra, A.Y. (2003). 'Virtual water flows between nations in relation to trade in livestock and livestock products', Value of Water Research Report Series No. 13, UNESCO-IHE.

Hoekstra, A.Y. and Hung, P.Q. (2002) 'A quantification of virtual water flows between nations in relation tointernational crop trade', Value of Water Research Report Series No. 11, UNESCO-IHE Institute for Water Education, Delft, the Netherlands.

Mekonnen, M.M. and Hoekstra, A.Y. (2010).The green, blue and grey water footprint of farm animals and animal products, Value of Water Research Report Series No.48, UNESCO-IHE.

Nayanarasi, H.A.D. and Atapattu, N.S.B.M. (2008) Effect of vitamin B or C enriched water on gastric transit time of fear-stressed layer chicken. (2008) Proceedings of the National Symposium, Faculty of Agriculture, University of Ruhuna.23rd and 24the Oct 2008. Pp 61

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Effects of foliar applications of different growth promoting substances on growth and yield of Bitter gourd (*Momordica charantia* L.)

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Abstract: An experiment was conducted to investigate the influence of different growth promoting substances on growth and yield of bitter gourd. Experiment was laid out in a randomized complete block design (RCBD) with four replications. Different foliar sprays viz. T1- Effective Microorganism (EM), T2- Cow urine, T3- Commercial growth promoting substance (Boom flower) and T4- Water (control) were tested. Highest values for average vine length (175.4 cm) and vine weight (40.9 g) were recorded in T3 and lowest male: female flower ratio (7:1) was obtained in T1. Maximum fruit length (29.9 cm) and fruit weight (158.1 g) were recorded in T1. The results revealed that application of EM is one of the ways to increase the yield of bitter gourd.

Keywords: Bitter gourd, foliar application, phyllosphere, yield

Introduction

Bitter gourd (*Momordica charantia* L.) is an important functional food crop grown in low and mid country during both *Maha* and *Yala* seasons in Sri Lanka. Bitter gourd is eaten as a vegetable and it has been successfully grown in elevation from mean sea level to about 1200 m in Sri Lanka. It has the highest nutritive values especially ascorbic acid and iron (Behera, 2004). It also has a compound known as charatin which is used in the treatment of diabetes to lower blood sugar levels (Shetty *et al*., 2005). In cucurbits male flowers are found to bloom at the lower nodes and female flowers appear a week later and never bloom first before the male flowers (Sumpoudlek and Abella, 1974). As a crop bitter gourd has a number of problems viz. low seed germination, small and "D" shaped fruit, low yield, non-synchronous flowering and diseases (Sikder, 2004).Use of plant growth regulators (PGRs) may be a useful alternative to increase the crop production. Globally PGRs have been used widely in crop production as it has significant positive effect on crop production. PGRs are being used to enhance the yield (Nickell, 1982).

EM is a certain harmonious grouping of three basic components such as photosynthetic bacteria, lactic acid bacteria, and yeasts. EM helps to increase the overall vigor and species diversity of the beneficial micro flora in the soil or whatever environment where EM is applied. Use of EM has spread to millions of households and thousands of farms around the world. Cow urine is a biological excretory product which being an antioxidant and a bio enhancer, increases the production of antibiotics and promotes healing processes. It gives hormones, enzymes, mineral salts, amino acids and also enriched in cytokines. Boom flower is a commercial product. It has been used as plant growth regulator and booster. It induces profuse flowering and helps in the retention of flower and fruits. No systematic study has been carried out to test the effect of these growth regulators on growth and yield of bitter gourd. Hence the present study was undertaken to find out the effect of foliar application of different growth promoting substances on growth and yield of bitter gourd.

Materials and Methods

The experiment was carried out at crop farm, Eastern University, Sri Lanka. The field is located in the latitude of 7° 43' N and the longitude of 81° 42' E. It belongs to the agro- ecological region of low country dry zone in Sri Lanka. The mean annual rainfall ranges from 1400 mm to 1680 mm and temperature varies from 30° to 32° C. The soil type is sandy regosol. The bitter gourd (*Momordica charantia* L.) variety Thirunelveli white was planted. The experiment was laid out in a randomized complete block design (RCBD) and treatments were replicated four times. Plants were raised in a pandal system, having 2.5m × 1.5m dimensions. Foliar sprays used viz. EM (T1), cow urine (T2), boom flower (T3) and water as control (T4). All the foliar solutions were diluted 10 times. Diluted EM, cow urine, boom flower and water (control) were applied at 30 and 60 days after planting (DAP). Fertilizers such as Urea, Triple Superphosphate and Muriate of Potash were applied at the rate of 225, 200 and 180 kg/ha respectively. All other management practices were done as recommended by the department of agriculture. Five vines were selected randomly from each plant at 45 DAP for the measurements of vine length and fresh weight of vine. The fruits were picked at 5 days interval. The parameters such as average length of vine, dry weight of vine, male: female flower ratio, length and fresh weight of fruits were measured. Data were analyzed by using SAS version 9.1 and treatment comparisons were performed by using Turkey's test at 5% significant level.

Results and Discussion

Length and weight of vine

Application of different foliar sprays had a significant ($p<0.05$) influence on average vine length and weight (Tab.1). Boom flower application produced highest vine length and weight of 175.4 cm and 40.9 g respectively compared to control (127.5 cm and 17.4 g respectively). Plant height increment was due to cell division, cell expansion and cell elongation. EM could significantly enhance the growth, yield and quality of crops (Higa and Wididana, 1991). EM treatment of Le Conte pear tree had significantly increased the vegetative growth, the number of current shoot/main branch, shoot length and diameter and leaf area (Abd-E1-Messeih *et al*., 2005). Commercial Boom flower consists of 2.2 % (w/v) of nitrogen. Boom flower has been used in crop production as energizer and yield booster. Application of boom flower quickly enters into the plant and changes the bio chemical pathways of plant to uptake more nutrient from the soil. It could be attributed to higher values of vine length and weight.

Tab.1: Effect of different growth promoting substances on average vine length and weight of bitter gourd

Treatments	Length of vine (cm)	Weight of vine (g)
T1	144.1 ^{c}	30.5 ^{b}
T2	152.6 ^{b}	34.7 ^{b}
T3	175.4 ^{a}	40.9 ^{a}
T4	127.5 ^{c}	17.4 ^{c}
F- test	*	*

*= Significant at 5% level of probability. Mean values in a column having the dissimilar letters indicate significant differences at 5% level.

Length and weight of fruits

Application of EM, Cow urine and Boom flower had effect on length and weight of fruit compared to control treatment (Tab.2). Significant ($p<0.05$) increase in fruit length and weight was observed in EM application compared to other treatments. Application of EM had produced highest fruit length (29.9 cm) and fruit weight (158 g) than other treatments. Shortest length (12.4 cm) and lowest weight of fruit (65.7 g) were obtained in control (Tab.2).

Tab.2: Effect of different growth promoting substances on average fruit length and weight of bitter gourd

Treatments	Length of fruit (cm)	Weight of fruit (g)
T1	29.9 ^{a}	158.1 ^{a}
T2	17.1 ^{b}	97.3 ^{b}
T3	14.5 ^{c}	86.4 ^{c}
T4	12.4 ^{d}	65.7 ^{d}
F- test	*	*

*= Significant at 5% level of probability. Mean values in a column having the dissimilar letters indicate significant differences at 5% level.

Auxins and a number of plant growth regulators are known to cause physiological modifications in plants mainly on flowering behavior, sex ratio, increased fruit set, enlargement and development of fruits, and source-sink relation. Growth regulators bring certain changes in metabolism during fruit and seed development. This might have caused greater accumulation of food reserves resulting in higher yield (Gedam *et al*., 1998; Rafeekher *et al*., 2002). Foliar application of N-fixing microorganisms to the phyllosphere of crop plants markedly increased their yield (Sen Gupta *et al*., 1982a; Sen Gupta *et al*., 1982b).

Male: female flower ratio

Influence of different growth promoting substances on male: female flower ratio is given in fig.1. The ratio was lower when the plants were treated with EM spray (7:1) and followed by plants treated with boom flower (8:1). The highest ratio (20:1) was recorded in control treatment.

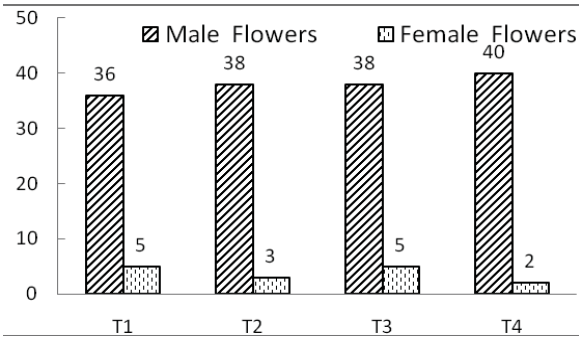

Fig. 1: Effect of different foliar applications on male: female flower ratio of bitter gourd

Application of growth promoting substances had influenced on the ratio of male and female flowers. Application of EM improves crop growth and yield by increasing photosynthesis, producing bioactive substances such as hormones and enzymes, controlling soil diseases and accelerating decomposition of lignin materials in the soil (Higa, 2000). Iwahori *et al*. (1970) reported that several growth regulators such as ethrel ethylene had enhanced female sex expression in cucurbits. Surendranath and Rao (1981) stated that the ratio of male and female flowers is determined by a balance of auxin and gibberellin; the balance in favour of auxin resulting in the formation of female and the latter of male flowers. It is suggested that certain microorganisms in EM culture including photosynthetic bacteria and N-fixing bacteria can enhance the plants' photosynthetic rate and efficiency, and N-fixing capacity as well (Pati and Chandra, 1981).

Conclusion

From the results, it is suggested that boom flower spray had significant effect on length and weight of the vine. Though, application of EM markedly increased the fruit weight and number of female flower compared to other growth promoting substances. These benefits may be achieved via changes in metabolisms during fruit setting and development. However, further studies are needed in order to confirm the effect of application of EM solution on bitter gourd.

References

Abd-E1-Messeih,W., Elseginy, M. and Kabeel, H. (2005). Effect of EM biostimulant on growth and fruiting of Le Conte pear tree in newly reclaimed area. *Alexandria Science Exchange Journal*, 26 (2): 121-128.

Behera,T. K. (2004). Heterosis in bitter gourd. *Journal of New Seeds*, 6: 217-222.

Gedam, V. M., Patil, R. B., Suryawanshi, Y. B and Mate, S. N. (1998). Effect of plant growth regulators and boron on flowering, fruiting and seed yield in bitter gourd. *Seed Research*. 26: 97-100.

Higa,T. and Wididana., G. N. (1991). Changes in the soil micro flora induced by Effective microorganisms. pp.153-162. In J.F. Parr, S.B. Hornick, and C.E. Whitman (ed.) Proceedings of the First International Conference on Kyusei Nature Farming. U.S. Department of Agriculture, Washington, D.C., USA.

Higa, T. (2000).What is EM Technology? EM *World Journal*, 1: 1-6.

Iwahori, S. J., Lyons, J. M. and Smith, O.E. (1970). Sex expression in cucumber plants as affected by 2-

chloroethyl phosphoric acid, ethylene and growth regulators. *Journal of Plant Physiology*, 46: 412-415.

Nickell, L. G. (1982). Plant Growth Regulators: Agricultural Uses. Springer Verlag, Berlin. Verlag Berlin-Heidelberg-New York 1982. 173 Seiten, 29 Abb., DM 47,50. Z. Pflanzenernaehr. Bodenk., 146: 128. doi: 10.1002/jpln.19831460115.

Pati, B. R. and Chandra, A. K. (1981). Effect of spraying nitrogen-fixing phyllosphere bacterial isolates on wheat plants. *Plant and Soil,* 61:419-427.

Rafeekher, M., Nair, S. A., Sorte, P. N., Hatwal, G. P. and Chandhan, P. M. (2002), Effect of growth regulators on growth and yield of summer cucumber. *Journal of Soils and Crops* 12:108-110.

Sen Gupta, B., Nandi, A. S. and Sen, S. P. (1982a). Utility of phyllosphere nitrogen-fixing microorganisms in the improvement of crop growth I. Rice. *Plant and Soil,* 68: 55-57.

Sen Gupta, B., Nandi, A. S. and Sen, S. P. (1982b). Utility of phyllosphere nitrogen-fixing microorganisms in the improvement of crop growth II. Wheat. *Plant and Soil*, 68: 69-74.

Shetty, A. K., Kumar., G. S., Sambaiah, K. and Salimath., P. V. (2005). Effect of bitter gourd (*Mommordica charantia* L.) on glycaemic status in steptozotocin induced diabetic rats. *Plant Foods for Human Nutrition*, 60: 109-112.

Sikder, B. (2004) Improvement of bitter gourd (*Momordica charantia* L.) through breeding and biotechnology. Ph.D thesis. Department of Genetics and Breeding, University of Rajshahi, Bangladesh.

Sumpoudlek, W. and Abella, P. A. (1974). Effect of ethrel on sex expression and yield of cucumber. *The CLSU. Science Journal*, 10(1): 22-27.

Surendranath, K. and Rao, T. S. (1981). Influence of growth regulators on sex expression and sex ratio in cucumber (*Cucumis sativus* L.). *The Andhra Agricultural Journal*, 28 (3 and 4):127-128.

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Effects of Cinnamon (Cinnamomum zeylanicum) Bark Powder on Growth Performance, Carcass Fat and Serum Cholesterol Levels of Broiler Chicken

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Abstract: Objective of this study was to determine the effects of dietary cinnamon bark powder (CNPW) on growth performance, carcass fat and serum cholesterol levels of broiler chicken. Giving a completely randomize design, ninety broiler chicks in 30 pens received broiler finisher diets containing either 0% (control), 0.1, 0.2, 0.3 0.4 or 0.5% CNPW *ad libitum* from day 23-43. Dietary CNPW tend (p=0.09) to increase the feed intake and feed conversion ratio (FCR) but had no effects on final live weight, weight gain, visceral organ weight, and gizzard, cloaacl and total fat contents or serum cholesterol level. Dietary CNPW at 0.1 and 0.4% increased the abdominal fat content compared to control. It was concluded that dietary CNPW used has no growth promoting or fat reducing effects in broiler chicken.

Keywords: Broiler, cinnamon, growth, fat, cholesterol

Introduction

With the ban on the use of antibiotic as growth promotants, poultry industry is looking for alternatives. Meanwhile, due to the health risks associated with animal fat, consumers demand carcasses and poultry meat with less fat contents. In these circumstances, search for safe growth promotants and carcass modifiers has become a priority research area. Herbs, spices, and various plant extracts have been received particular attention as possible alternatives to antibiotic growth promotants (AGP), since they are considered natural products (Henandez *et al*., 2004). A range of phytogenic feed additives including thyme (*Thymus vulgaris*), clove (*Syzygium aromaticum*), turmeric (*Curcuma longa*), black pepper (*Piper nigram*), oregano (*Oregano vulgar*), garlic (*Alum sativa*), cinnamon (*Cinnamomom ceylanicum*), and Fenugreek (*Trigonella foenum graecum*) have been studied as phytogenic feed additives in poultry production.

Cinnamon (*Cinnamomum* zeylanicum) is a valued spice used all over the world. *C zeylanicum* is indigenous to Sri Lanka. In Ayurvedic and ethno-medicine various parts of the cinnamon are widely used. The main chemical constituents of Cinnamon in cynamaldehyde and euginol. Recent studies showed cinnamon powder, cinnamaldehyde alone or in combination with other essential oils have a wide array of beneficial effects in poultry. Some of those effects include increased feed intake (Al-Kassie 2009), improved performance and feed efficiency (Isabel and Santos,2009; Al-Kassie 2009 and Kamel, 2001), increased pancreatic and intestinal lipase activity (Kim *et al.,* 2010), increased breast meat yield (Isabel and Santos, 2009), improvement in health status (Al-Kassie, 2009 and Kamel 2001), protection against pathogens such as *Escherichia coli*, *Pseudomonas aeruginosa, Enterococus faecalis, Staphylococcus aureus, Staphylococcus epidermis*, *Salmonella sp*. *Helicobacter pylori* and *Parahemolyticus* (Chang *et al*., 2001 and Taback *et al*., 1999). Even in Sri Lanka, the world highest cinnamon producing country, cinnamon oil and cinnamaldehyde are too expensive to be used as a feed additive. Since cinnamon bark is relatively cheap and easily available, the present experiment evaluated the growth promoting and fat reducing effects of CNPW in broiler chicken.

Materials and Methods

Table 1. Ingredient composition and calculated nutrient contents of the basal diet

Ingredient	g/kg
Yellow maize	588
Rice bran	62
Soya bean meal	249
Coconut oil	35
Fish meal	39.5
DCP	12.3
$CaCo_3$	9
D Methionine	1
Salt	2
Vit min mix	2
Calculated nutrient contents	
CP %	199
ME (Kcal/kg)	3 106
Ca	9.0
Non phytase phosphorus	3.5
Lysine	11
Methionine + Cystein	7.7
Methionine	3.6
Crude fibre	37.7

Chicks were brooded on an electrical brooded for 10 days. Until day 22, Chicks were fed a commercial broiler starter diet. On day 22, 90 chicks were allocated into 30 floor pens (70cmx70cmx75cm) so that live weight variation among the pens are minimum. Pens were randomly allocated into replicates of five dietary treatments. Each pen had a feeder and a drinker. From day 23-43, birds were fed one of the five experimental diets containing either 0 (control), 0.1, 0.2, 0.3, 0.4 or 0.5% cinnamon bark powder *ad libitum*. Cinnamon bark was purchased from a local cinnamon peeler and ground. Except for energy (3100 Kcal ME/kg), control diet met the nutrient requirements as set out in NRC (1994) (Table 1). Daily feed and water intakes were taken. Serum cholesterol contents of six randomly selected birds from each pen were determined using a commercial assay kit (SPINREACT, S.A. Spain), on day 42. One randomly selected bird from each pen was killed by cervical dislocation on day 43 and dissected to determine internal organ weights and gizzard, abdominal, cloacal and total fat contents of the carcass. Data were analyzed as a completely randomize design with six replicates, using SAS. Significant means were compared using DMRT procedure.

Results and Discussion

Live weight on day 43 was not affected by dietary CNPW. Feed intakes of the broilers fed CNPW were tend to be (p=.0.09) higher than that of control group (Table 2). However, improved feed intake did not improve the FCR. The best FCR was reported by the birds fed CNPW free control diet. Furthermore, FCRs of the broilers fed CNPW were tend to be higher than the control group. While maintaining the weight gain more or less similar across the treatment, increased feed intake resulted in higher FCRs and vise versa. This observation clearly suggests that dietary CNPW used in this experiment had no positive effects on digestion and absorption process and/or nutrient utilization efficiency as shown by a number of other studies (Kamel, 2001) and Hernandez, et al. (2004).

In general, performance parameters of this study are contradictory to those of Al-Kassie, (2009) and Lee et al. (2004) who found improved performance in broilers fed diets supplemented with Cinnnamaldehyde. However it must be noted that both of the above studies used pure cinnamaldehyde whereas the present study used cinnamon powder. The effects of a feed additive depend on a range of factors including it's dose and the duration of the treatment. It may be a possibility that the CNPW levels or the duration of the feeding might not have been the optimum.

1. As a percentage of empty carcass Park (2008) showed that 3% but not 2 or 4 or 5% dietary CNPW improved the performance in broiler chicken. Meanwhile, Toghyani, (2011) has reported that 0.2 % dietary CNPW improved the performance of broilers when fed at least for four weeks. Feeding of higher levels of CNPW such as 2% may not be financial feasibility. Therefore, further research are needed to determine growth promoting effects of lower dietary levels given for longer period.

None of the visceral organ weights was affected by the dietary CNPW levels. Toghyani, (2011) Barreto et al. (2008) and Hernandaze et al. (2004) have also shown that dietary CNPW had no effects on internal organ weights.

Even though there was no effect on the cloacal, gizzard and total fat contents, the abdominal fat contents were affected by the dietary CNPW. Similarly, Rasika and Atapattu, (2012) have reported that dietary curry leaf powder had different effects on the fat deposition in different regions of the broiler carcass. However, the dietary CNPW levels used in this experiment increased the abdominal fat content. The abdominal fat content was lowest when birds were fed CNPW free diet. Meanwhile, birds fed 0.1% CNPW gave significantly higher abdominal fat content than those fed 0, 0.2 and 0.3 dietary CNPW fed birds. In contrast to above findings, Isabel and Santos (2009) have found no effect of feed additive containing cinnamaldehyde on abdominal fat contents. Interestingly, Though not significant, the total fat content of the broilers fed dietary CNPW was higher than control group

Rasika and Atapattu (2013) have also shown that dietary curly leaf powder at, 0.5, 1, 1.5 and 2% increased the total fat contents of the broiler carcass, compared to control birds.

Therefore, results of this experiment suggest that CNPW levels used in this experiment had no fat lowering effects in broiler chicken. However, as in the case with performance parameters, it would be interesting to investigate whether other doses and treatment durations have favorable effects on carcass fat.

In contrast to the findings of Ak-Kessie, (2009) the serum cholesterol levels were also not affected by the dietary CNPW.

It was concluded that dietary CNPW used in this experiment had no growth promoting or fat lowering effects in broiler chicken.

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References

Al-KASSIE, G. A. (2009) Influence of Two Plant Extracts Derived from Thyme and Cinnamon on Broiler Performance. *Pakistan Veterinary Journal*, 29(4), pp. 169-173

BARRETO, M. S. R. MENTEN, J. F. M. RACANICCI, A. M. C. PEREIRA, P. W. Z. and RIZZO, P. V. (2008). Plant Extracts Used as Growth Promoters in Broilers, .Revista *Brasileira de Ciência Avícola*, 10(2), pp. 109-115.

CHANG, S. T. CHEN P. F. and Chang S. C., (2001) Antibacterial Activity of Leaf Essential Oils and Their constituents from Cinnamomun Osmophloeum. Journal of *Ethnopharmacology*., 77, pp. 123-127

HENANDEZ, F. MARDRID, J. GARCIA, V. ORENGO, J. and MEGIAS, M.D. (2004) Influence of Two Plant Extracts on Broiler Performance, Digestibility, and Digestive Organ Size. *Poultry Science*, 83, pp.169-174

HERNANDEZ, F. MADRID, J. GARCIA, V. ORENGO, J. and MEGIAS, M. D. (2004) Influence of Two Plant Extracts on Broilers Performance, Digestibility, and Digestive Organ Size. *Poultry Science*, 83(2), pp. 169-174.

ISABEL, B. and SANTOS, Y. (2009) Effects of Dietary Organic Acids and Essential Oils on Growth Performance and Carcass Characteristics of Broiler Chickens. The Journal of *Applied Poultry Research*, 18(3), pp. 472-476..

KAMEL, C. (2001). Tracing Modes of Action and the Roles of Plant Extracts in Non-Ruminants. In: P. C. Garnsworthy and J. Wiseman, (eds.). *Recent Advances in Animal Nutrition*, Pp. 135–150 in Nottingham University Press, Nottingham, UK

KIM, D. K. LILLEHOJ, H. S. LEE, S. H. JANG, S. I., and BRAVO, D. (2010) High-Throughput Gene Expression Analysis of Intestinal Intraepithelial

Lymphocytes after Oral Feeding of Carvacrol, Cinnamaldehyde, or Capsicum Oleoresin. *Poultry Science*, 89(1), pp. 68-81.

LEE, K. W. EVERTS, H. H. KAPPERT, J.J. WOUTERSE, H. FREHNER, M and BEYNEN, A.C. (2004) Cinnaman- Aldehyde, but not Thymol, Counteracts the Carboxymethyl Cellulose-Induced Growth Depression in Female Broiler Chickens. *International Journal of Poultry Science*, 3, pp. 608- 612

NRC (1994). Nutrient Requirements of Poultry. National Research Council National Academy of Science, Washington, D.C

PARK, B. S. (2008) Effect of Dietary Cinnamon Powder on Savor and Quality of Chicken Meat in Broiler Chickens. *Journal of The Korean Society of Food Science and Nutrition*, 37.pp.618-624.

RASIKA, S. H.K. and ATAPATTU N.S.B.M. (2012) Effects of Curry Leaf Powder on Growth Performance, Carcass Fat and Serum Cholesterol Levels of Broiler Chicken. In: Proceedings of the International Symposium on Agriculture and Environment, Student Session. 22nd Feb 2013, University of Ruhuna, Pp. 14-17.

TABACK, M., R. Armon and I. Neeman, 1999. Cinnamon extracts inhibitory effect on Helicobater pylori. *Journal of Ethnophamacology*., 67: 269-277.

TOGHYANI, M. TOGHYANI, M. GHEISARI, A. GHALAMKARI, G. and EGHBALSAIED, S. (2011) Evaluation of Cinnamon and Garlic as Antibiotic Growth Promoter Substitutions on Performance, Immune Responses, Serum Biochemical and Haematological Parameters in Broiler Chicks. *Livestock Science*, 138(1), pp. 167-173.

Table 2
Effects of dietary cinnamon powder on growth performance, visceral organ weight, carcass fat and serum cholesterol contents of broiler chicken

Parameter	Level of dietary cinnamon						Pooled SEM	p value
	0	0.1	0.2	0.3	0.4	0.5		
Feed intake (g)								
23 - 43d	2144 ^{b}	2575 ^{a}	2338 ^{ab}	2573 ^{a}	2423 ^{ab}	2228 ^{ab}	122.44	0.09
Live weight (g)								
23d	817	812	797	808	827	791	17.48	0.73
43d	2101	2140	2090	2172	2237	2106	60.14	0.51
Weight gain (g)								
23 - 43d	1284	1328	1292	1363	1410	1314	56.35	0.62
Feed conversion ratio								
23 - 43d	1.67 ^{b}	1.93 ^{a}	1.82 ^{ab}	1.89 ^{ab}	1.72 ^{ab}	1.69 ^{ab}	0.08	0.09
Carcass parameters [1]								
Dressing %								
	78.5	84.33	75.66	81	78.83	82.5	3.00	0.39
Gizzard %								
	2.8	2.2	3.03	2.55	2.55	2.4	0.28	0.34
Liver %								
	2.65	3.08	3.17	3.06	3.05	2.85	0.25	0.71
Pancreas %								
	0.22	0.21	0.24	0.21	0.19	0.2	0.02	0.56
SI weight (g)								
	3.68	3.84	3.72	4.39	4.26	4.06	5.72	0.66
SI length								
	11.65	10	11.42	11.15	9.31	10.41	9.66	0.31
Gizzard fat %								
	0.97	1.12	1.46	2.55	1.03	0.72	0.22	0.27
Abdominal fat %								
	0.20 ^{c}	0.43 ^{a}	0.27 ^{bc}	0.28 ^{bc}	0.37 ^{ab}	0.33 ^{abc}	0.04	0.01
Cloacal fat %								
	1.81	2.23	2.11	2.07	2.12	2.31	0.21	0.66
Total fat %								
	2.99 ^{a}	3.79 ^{a}	3.85 ^{a}	3.20 ^{ab}	3.53 ^{ab}	3.37 ^{ab}	0.24	0.13
Serum cholesterol (mg/dl)								
	121	115	125	129	118	127	13.63	0.45

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Morphological variability, Germination ability and Survival rate of Weedy rice seeds in Ampara and Matara districts in Sri Lanka.

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Abstract: High diversity in morphology and physiology of weedy rice is important resource for rice breeding. Present study was aimed to determine the seed morphological diversity, germination percentage and survival rate of weedy rice populations in Matara and Ampara districts. Significant diversity of seed shape, awn length, hull color and pericarp color was observed. Germination percentage and survival rates were highly variable. Our results showed that great diversity in weedy rice seeds and the favorable characteristics such high germination percentage, high survival ability, own less seeds, proper seed shape and pericarp color can be incorporated into cultivated rice varieties in rice breeding programs.

Keywords: weedy rice, seed morphology, germination ability, survival rate

Introduction

Weedy rice (*Oryza sativa* complex) is a conspecific weedy relative of cultivated rice (*Oryza sativa* L.) that occurs in rice fields' worldwide (Michael *et al.*, 2010). In mid1990's, weedy rice was first identified as a threat from Vavunia, Ampara, and Batticaloa districts (Marambe and Amarasinghe, 2000) and at present that is most common in all rice growing area in Sri Lanka. The superior competitive ability of weedy rice over cultivated rice has contributed to its rapid spread in the country (Abeysekera *et al.*, 2010). Weedy rice has close affinity with cultivated rice, in terms of morphological and physiological characteristics (Fogliatto *et al.,* 2011). This similarity has even led weedy rice to be classified as the same species as cultivated rice (Vaughan *et al.*, 2001). Morphologically, weedy rice is highly variable in almost all the vegetative and reproductive characteristics with each other and appears to be an intermediate between wild and cultivated rice (Cao*et al.*, 2006). Weedy rice is considered as a useful germplasmas it has successfully adapted to the natural growing conditions (Heu *et al.*, 1990). It also has many useful genes for cold tolerance (Chang *et al.,* 2004), grain quality and germination characteristics (Ma *et al.*, 2008) and high salinity and drought tolerance (Jiang *et al.,*1985). Morphological traits such as seed color and seed shapes and own characters are highly variable in weedy rice and physiological traits such as degree of dormancy, germination ability, viability and longevity also showed high variability. Seed traits of white pericarps, own less, non-dormant, higher survival rates are useful characteristics for crop improvement in rice breeding. Therefore, this study was aimed to determine the morphological diversity, germination percentage and survival rate of selected weedy rice populations. The information gathered in this study may be useful in rice improvement programs in the future.

Material and methods

An extensive field survey was carried out at Ampara and Matara districts in Sri Lanka and total of 06 weedy rice infested locations were selected intentionally as sampling sites. The selected sites were Akuressa, Thihagoda and Mulatiyana from Matara district and Akkareipattu, Ampara and Lahugala from Ampara district. Panicles from 30 individuals were collected from each location. Seed morphology was recorded by

playing attention to awn traits, seed shape, and seed size and pericarp pigmentation. The collected mature seeds were dried to 14% of moisture and 10 weedy rice seeds were randomly collected from each panicle from each population, mixed well and randomly collected100 seeds were used for germination test with four replicates. Weedy rice seeds were soaked in water for 24 hours, covered for 24 hours by cloth bag (standard germination percentage testing method) and they were put on the humid filter paper in the petri dish with sufficient light. The numbers of germinated seeds were counted (as seeds had radical appearing). Germinated seeds were transferred into mud trays and healthy plants were counted after 21 days. Survival rate was assessed the percentage of germinated seeds.

Results

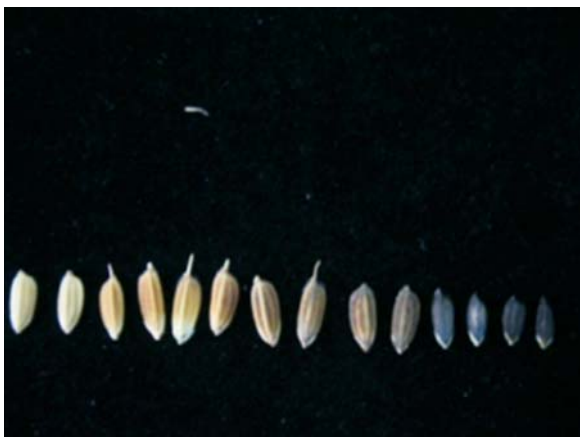
Seed morphological diversity

Our results showed that there is a great diversity in seeds in terms of seed shape, awn length, hull color and pericarp color.

Weedy rice seed collected from Matara and Ampara districts showed great variation for the own length which varied from 0 (ownless) to 10 cm (Fig.1a). A considerable diversity was observed in seed shape (Fig.1b) andhull color which varied from pale white to black (Fig.1c). Pericarp color varied from white to brownish red (Fig.1d). This variability observed among populations as well as within the populations.

a. Variation in own length

b. Variation in seed shapes



c. Variation in hull color

d. Variation in pericarp color

Fig.1. Morphological diversity of weedy rice seeds collected from Ampara and Matara districts in 2012.

Table 01:
Percentages of various categories of seed morphological traits of weedy rice collected from Matara and Ampara districts in 2012.

Population	Hull color			Pericarp color			Own length			Seed shape		
	Straw	Black	Gray	Red	Brown	White	Long	Medium	Own less	Long	Intermediate	Round
Akuressa	60%	10%	30%	88%	8%	4%	25%	35%	40%	30%	45%	25%
Thihagoda	70%	5%	25%	80%	15%	5%	22%	26%	52%	32%	43%	25%
Mulatiyana	62%	13%	25%	90%	6%	4%	18%	42%	40%	25%	55%	15%
Ampara	50%	22%	28%	78%	18%	4%	30%	25%	45%	45%	43%	12%
Akkaraipattu	56%	15%	29%	83%	11%	6%	35%	25%	40%	48%	38%	14%
Lahugala	63%	16%	21%	81%	12%	7%	45%	25%	30%	55%	35%	10%

With reference to the all populations straw hull color seeds occur in a higher percentage than black and gray hull color seeds. Red pericarp color seeds occur in a higher percentage (>80%) while white pericarp seeds occur in a lower percentage (<7%). Own less seeds has considerable percentage (>35%) in the all populations

Germination percentage was highly variable in weedy rice populations in Ampara and Matara districts. Ampara population showed highest germination percentage (89%) while Lahugala population showed lowest germination percentage (7%). The populations showing high germination percentages would be useful for plant breeding programs.

Germination ability

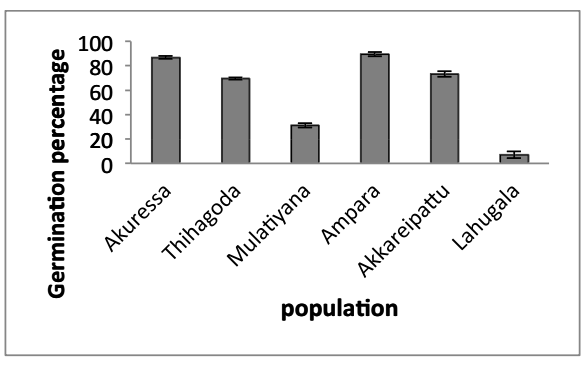


Fig.2: Germination percentage of weedy rice populations collected from Ampara and Matara districts in 2012.Vertical bars on the columns showed standard errors.

Survival rates

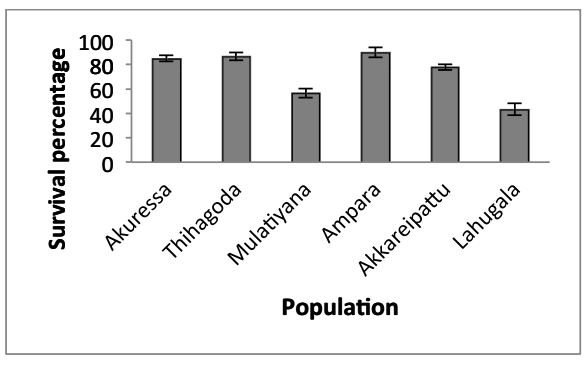


Fig.6: Survival percentage of weedy rice populations collected from Ampara and matara districts in 2012.

Survival percentage was highly variable in weedy rice population in Ampara and Matara districts. Ampara population showed highest survival percentage (90%) while Lahugala population showed lowest survival percentage (43.33%). Except Lahugala and Mulatiyana populations other populations showed high survival rates.

Discussion

There was a great diversity in seed morphological traits such as seed shape, own length, hull color and pericarp color within and among populations. Previous studies have reported that diversity of seed traits such as hull color in weedy rice is greater than cultivated rice (Fogliatto*et al.*, 2011). Constantin (1960) reported three types of red rice based on hull color. Further, straw hull red rice is more common than black hull (Huey, 1978; Smith, 1981). In this study we mainly observed hull color of red brown that is with the agreement of Prathepa (2009). Morphological and topographical characteristics of plant organs such as the shape and size of seeds and the structure of incidental features have been useful weapons in identifying and classifying the plant and weed species (Noda *et al.*, 1985). Own less seed is an improved traitand high diversity in seed shapes and pericarp color may be important for developing quality rice to meet diverse consumer demand. Populations with higher germination percentages (more than 80%) and higher survival ability (more than 80%) are important characteristics for crop improvement. Awn length and distribution, seed length, thousand seed weight and germination rates were the most important traits influencing the variability among populations (Fogliatto*et al.*, 2011). The morphological diversity observed, not only among the weedy rice morphotypes but also within them, offers an array of traits that could be studied and incorporated to future rice-breeding programs (Griselda *et al.*, 2004). Our results showed that great diversity in weedy rice seeds and the favorable characteristics such as high germination percentage, high survival ability, own less seeds, proper seed shape and pericarp color can beincorporated into cultivated rice varieties in rice breeding programs. In addition, proper understanding on seed germinability and survival is crucial for adopting efficient management practices.

References

Abeysekara, A.S.K. Herath, H.M.S. Wickrame, U.B. Nugaliyadde, L. and Johnson, D.E., (2010). Germinability, viability, and longevity of weedy and cultivated rice in Sri Lanka.Presented at the International Rice Research Conference, 8-12 November 2010, Hanoi, Vietnam.

Cao, Q.J. Xia, H.Rong, J. Sala, F. Spada, A. and Grassi F., (2006). Genetic Diversity and Origin of Weedy Rice (*Oryza sativa* f. *spontanea*) Populations Found in North-eastern China Revealed by Simple Sequence Repeat (SSR) Markers. *Journal of Annals of Botany*.98(6) p.1241–1252.

Chang, S.O. Yong, H.C, Seung, J.L. Dong, B.Y. Huhn, P.M. and Sang, N.A., (2004). Mapping of quantitative trait loci for cold tolerance in weedy rice.*Breed. Sci.* 54.P.373–380.

Constantin, M.J., (1960) Characteristics of red rice in Louisiana. Ph.D. Dissertation, Louisiana State Univ. and Agric.And Mechanical College, Baton Rouge, La.

Fogliatto, S., Vidotto, F., and Ferrero, A., (2011).Morphological characterization of Italian weedy rice(*Oryza sativa*) populations.*An International journal of Weed Biology, Ecology and Vegetation management*.P.60-69.

Griselda, A. Elena, S. Sergio, V. Jorge, L. Tania, Q. And Espinoza M., (2004).The weedy rice complex in Costa Rica. I. Morphological study of relationships between commercial rice varieties, wild Oryzarelatives and weedy types. *Journal of Genetic Resources and Crop Evolution*.52. p. 575–587.

Heu, M.H. Cho, Y.C. and Suh, H.S., (1990). Cross affinity of Korean weedy rice to the cultivars. *Korean J. Crop Sci*. 35. p. 233–238.

Huey, B.A. and Baldwin, F.L., (1978).Red rice control.p.19-25 in E.F. Eastin(ed.) Red rice: Research and control.Texas Agric. Exp. Stn. Bull.B-1270.

Jiang, H. Wu, J.L. and Wang, G.L., (1985). Studies on Ludao of Lianyungang.*Crop Genet*. 2. p.4–7.

Ma, D.R. Wang, N. Wang, Y. Jia, D.T. and Chen, W.F., (2008). Germination dynamics of weedy rice in northern china at different sowing depths, Chin. J. Rice Sci. 22. p.215–218.

Marambe, B. and Amarasinghe, L. (2000). Weedy rice in Sri Lanka.In Wild and Weedy Rice in Rice Ecosystems in Asia-A Review.(Eds. B.B. Baki, D.V Chin, A.M. Mortimer), International Rice Research Institute, Philippines. p.79-82.

Michael, R.,Thurber, C.S. Gross, B.L. Olsen, K.M. Jia, Y.,andCaicedo, A.L., (2010). Genomic patterns of nucleotide diversity in divergent populations of U.S. weedy rice. *BMC Evolutionary Biology*.p.10.

Noda, K. Prakongvongs, C. and Chaiwiratnukul, L.,(1985). Topography of the seeds and leaves of tropical weeds – with a scanning electron microscope.*National Weed Science Research Institute Project*.p.158.

Prathepha, P., (2009). Seed morphological traits and genotypic diversity of weedy rice populations found in Thai Hom Mali rice fields of north-eastern Thailand. *Weed Biol. Manage*. p.1-9.

Smith, R.J., (1981). Control of red rice (*Oryza sativa*) in water-seeded rice (*O. sativa*).*Weed Sci.* 29. p.663-666.

Vaughan, L.K. Ottis, B.V. and Prazak-Havey, A.M., (2001). Is all red rice found in commercial rice really *Oryza sativa*? *Weed Science*. 49. p.468–476.

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Effects of the Withdrawal of Vitamin Trace Mineral Mixture from Broiler Finisher Diet Supplemented With Phytase on Growth Performance, Visceral Organ Weights and Feed Cost

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Abstract: Objective of this study was to determine the effects of the withdrawal of vitamin trace mineral mixture (VTM) from a broiler finisher diet supplemented with phytase on growth performance, visceral organ weights and feed cost. Giving a completely randomize design in 2 (0 or 1000 FTU phytase /Kg) * 2 (0 or 2.5 g VTM/kg) factorial arrangement, 128 broiler chicks received one of the four experimental diets ad libitum, from day 23-43. Withdrawal of VTM reduced feed intake, live weight and weight gain while increasing the relative weight of the visceral organs. Removal of VTM reduced the tibia length but had no effect on ash content. Costs per Kg empty carcass and Kg of seleable carcass were lowest when VTM free diet was supplemented with phytase. It is concluded that, despite inferior growth performance, withdrawal of VTM is financially profitable if the diet is supplemented with phytase supplemented.

Keywords: Vitamin, trace mineral, withdrawal, performance, broiler

Introduction

Poultry ration formulations are not normally balanced for trace minerals and vitamins. Instead, a vitamin/trace mineral mixture (VTM) is added to make sure that the ration contains sufficient levels of those nutrients. However, modern commercial feed formulators adopt wide safety margin and use 3-10 times higher trace mineral and vitamin level than recommended (Skinner et al. 1992; Inal et al., 2001). Under Sri Lankan condition, even added at recommended level (2.5g/kg), VTM incurs Rs 2.50 per kg of feed. Furthermore, since a higher proportion of these trace elements are not absorbed, use of excessive levels of trace minerals is not an environmentally sound practice.

Broilers vitamin and mineral requirements reduce as they mature. Also, when birds grow at slower rate due to high temperature and/or poor management conditions which is often the case in Sri Lanka, their vitamin and trace mineral requirements may be even low. Vitamin premix of a broiler finisher diet can be removed when grown on litter which is a good source of many vitamins and minerals (Shahrasb et al. 2012). Mash form diets which have not been subjected heat processing contains a substantial level of vitamins. Therefore, even without a VTM, a typical poultry feed could meet a substantial level of trace minerals and vitamins requirements. It has also been shown that availability of minerals is increased when the diet is deficient in minerals. Above arguments have been supported by a number of studies (Khajali et al., 2006; Maiorka et al., 2002; Deyhim et al., 1993; Skinner et al., 1992) which have shown that VTM can be removed totally or partially from broiler diets, for a short period, particularly during later stages of growth without affecting performance. However, Sayadi et al. (2005) showed that removal of VTM for 21 days reduced the performance.

Supplementation of poultry diets is now a common practice. Apart from P, phytase improves the availability of Ca and a range of other trace minerals such as Zn Mg, Fe, Cu (Viveros et al., 2002). Objective of this study was to determine whether VTM of a broiler finisher diet could be removed for a longer period if the diet is supplemented with phytase.

Materials and Methods

Day old broiler chicks were brooded for 10 days and fed a commercial broiler starter diet until day 22. On day 22, 128 chicks were allocated in to 32 floor pens so that live weight variation among pens was minimum. Pens were randomly assigned into eight replicates of a completely randomized design in 2 x 2 factorial arrangement. Four broiler finisher diets with Phytaze or without phytase, each with or without vitamin/trace mineral mixture were prepared. Experimental factors were two dietary phytase (0 or 1000 FTU/Kg) and two dietary VTM levels (0 or 2.5g/Kg). Ingredient composition and the calculated nutrient composition of the control diet (phytase – VTM+) is given in Table 1. Phytazag was the phytase source and all rations had 3g of non phytate phosphorus (NPP)/kg. VTM free diet had 2.5g washed sand/kg. All diets fed *ad libitum* from day 23-43. Birds were weighed on day 33 and 43. One randomly selected bird from each pen was killed on day 43 and dissected to determine the carcass parameters. Left tibias were analyzed for fat free tibia ash. Breast meat samples were boiled for 30 minutes and subjected to organoleptic evaluation by 30 untrained panelists. Four organoleptic attributes (colour, taste, toughness and overall acceptability) were evaluated on a five point Likert scale.

5, Like very much 4, Like somewhat 3. Neither like no Dislike 2, Somewhat dislike 1, Don't like at all

Growth performance and carcass data were analyzed as a completely randomize design in 2 x 2 factorial arrangement. Significant main effects were compared using DMRT procedure. Organoleptic data were analyzed using kruskal Wollis procedure.

Table 1:
Ingredient composition and calculated nutrient composition

Ingredient	g/Kg	
Maize meal	507	
Rice bran	150	
Soya bean meal	249	
coconut oil	31	
Fish meal	35	
Dicalcium phosphate	8	
CaCo3	14.5	
D Methionine	0.5	
Salt	2.5	
Vit/min mix	2.5	0
Washed sand	0	2.5
Phytase	+/-	
Calculated nutrient composition		
CP	200	
ME (Kcal/kg)	3050	
Ca	10	
Non phyteen phosporus	3	
Lysine	10.7	
Methionine+Cystine	7.3	
Methionine	3.6	
Crude fibre	45.9	

Results and Discussion

Suggesting that withdrawal of VTM from day 23-43 had no severe negative effects on health of the birds, no mortalities was reported during the experiment. Apart from the improved FCR of young birds from day 23-33, phytase had no significant effect on any of the growth performance parameters (Table 2). Effects of supplemental phytase are more obvious when diet is deficient in NPP. The absence of positive effects of phytase on performance may be due to the use of marginally deficient (3g/kg) NPP level. Phytase*VTM withdrawal interaction had no significant effect on any of the growth performance or carcass parameters (Table 3). In contrast, withdrawal of VTM reduced feed intake, live weight and weight gain significantly. However, withdrawal of the VTM had no effect on FCR. In line with our results, a number of other studies (Siahpour *et al*., 2006; Sayadi *et al*., 2005 and Wang *et al*., 2008) have also shown that removal of VTM for 21 days decreased daily weight gain, feed intake and feed efficiency. However, 7-14 days of VTM withdrawal at later stages of the growth had no influence on weight gain, feed intake and feed efficiency (Siahpour *et al*., 2006; Maiorka *et al*., 2002). It seems that the effect of the withdrawal of VTM is

influenced by the time of withdrawal and the length of the withdrawal. Results of this experiment suggest that 21 days of VTM withdrawal from 23-43 impairs the performance, even with supplemental phytase.

As reviewed by Woyengo and Nyachoti, (2011), Phytase supplementation increased the tibia ash content. Meanwhile phytase reduced the relative weight of the liver and heart. Similarly, Viveros *et al.* (2002) have also reported that supplemental phytase reduced the relative weight of liver of broiler chicken. Interestingly, removal of VTM reduced the length of the tibia but had no effect on tibia ash content. Wang *et al.* (2008) have also shown that withdrawal of VTM had no effect on tibia ash content. The diets contained adequate level of Ca and 0.3% NPP. Since bones are mainly made up of Ca and P, a negligible effect on bone ash could be expected. It seems that trace minerals and vitamins have stronger effect on the elongation of the long bones than on the bone ash content.

Siahpour *et al.* (2006) found no effect of VTM withdrawal on carcass parameters. However, in the present experiment, withdrawal of VTM increased the relative weight of the visceral organs such as liver, heart, gizzard and proventriculus and the relative length of the small intestine. In partitioning the available nutrient within the body, supply organs such as digestive tract get priority. Several restricted feeding strategies (Rosenbrough *et al,*. 1988; Palo *et al.*, 1995; Atapattu and Lal, 2009) have shown that relative weights of digestive organs and heart increased when general growth is retarded. Therefore, increased relative weight of the visceral organs of the birds given VTM free diet may be due to the continued growth of those organs despite the general reduction of growth.

Interestingly, taste of the meat of the broilers fed diet supplemental phytase and VTM was significantly higher than the counterparts fed other three diets. Other organoleptic properties were not significantly different among the treatments.

Phytase supplemental incurred 0.40 Rs of an addition cost per kg of diet whereas withdrawal of VTM saved 2.50 Rs per Kg of feed. Feed cost per Kg of empty carcass or per Kg of seleable carcass were not significantly altered due to dietary manipulation studied (Table 4). However, costs per Kg empty carcass and Kg of seleable carcass was highest when there was

Table 2.
Effects of the withdrawal of vitamin trace mineral mixture from broiler finisher diet supplemented with phytase on growth performance

Treatment factors				Live weight (g)			Feed intake (g)			Weight gain (g)			FCR		
				23 d	33 d	43 d	23–33 d	34–43 d	23–43 d	23–33 d	34–43 d	23–43 d	23–33 d	34–43 d	23–43 d
Ph	+	VTM	+	918	1620	918	1201	1572	2774	701	682	1384	1.70	2.35	2.01
			–	890	1535	2302	1123	1337	2451	645	574	1219	1.74	2.42	2.01
	–		+	911	1590	2237	1204	1423	2627	679	646	1325	1.78	2.24	1.98
			–	903	1493	2052	1195	1298	2493	589	559	1149	2.03	2.37	2.18
SEM				23.6	53.9	96.4	111	114	174	50.5	80.3	92.1	0.14	0.31	0.11
Probability		Ph		NS	NS	NS	NS	NS	NS	NS	NS	NS	*	NS	NS
		VTM		NS	**	***	NS	**	*	**	*	***	NS	NS	NS
		Interaction		NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS
Main effects		Ph	+	904	1311	2206	1162	1360	2560	673	628	1301	1.72[b]	2.38	2.01
			–	907	1298	2144	1200	1450	2612	634	602	1237	1.90[a]	2.30	2.08
		VTM	+	915	1605[a]	2269[a]	1202	1497[a]	2700[a]	690[a]	664	1354[a]	1.74	2.29	1.99

reatment factors	Parameter1	Organoleptic properties

no supplemental phytase and VTM. On the other hand respective cost items were lowest when VTM free diet was supplemented with phytase. Contrast to our findings, Sayadi *et al.* (2005) showed that removal of VTM increased the feed cost per unit of product. Results of this experiment clearly show that despite the retarded growth performance, withdrawal of VTM is financially profitable if the diet is supplemented with phytase supplemented.

Table 3:
Effects of the withdrawal of vitamin trace mineral mixture from broiler finisher diet supplemented with phytase on visceral organ weight and meat organoleptic properties

				Gizzard	Liver	Heart	Proventriculus	S.Intestine (L)	Tibia length	Tibia ash (%)	Color	Taste	Toughness	Overall Acceptability
Ph	+	VTM	+	2.17	2.71	0.52	0.96	9.75	6.95	44.7	4.1	4.3[a]	3.9	4.1
			–	2.28	3.43	0.67	1.27	10.8	6.77	45.7	4.0	3.6[b]	3.6	3.7
	–		+	2.37	3.16	0.66	1.03	10.4	6.97	41.5	3.7	3.6[b]	3.5	3.7
			–	2.58	3.24	0.82	1.09	11.8	6.67	43.7	3.8	3.6[b]	3.5	3.7
SEM				0.37	0.38	0.12	0.12	1.05	0.22	2.28				
Probability		Ph		NS	*	*	NS	NS	NS	*				
		VM		NS	*	*	**	*	*	NS				
		Interaction		NS	NS	NS	NS	NS	NS	NS				
Main effects		Ph	+	2.23	3.07[b]	0.66[b]	1.11	10.30	6.86	45.2[b]				
			–	2.48	3.20[a]	0.74[a]	1.06	11.12	6.82	42.6[a]				
		VTM	+	2.27	2.94[b]	0.59[b]	1.00[b]	10.10[b]	6.96a	43.1				
			–	2.43	3.34[a]	0.74[a]	1.18[a]	11.32[a]	6.72b	44.7				

1. As a % of empty carcass

Means with different superscripts within a column are significantly different at $p<0.05$

Table 4:
Effects of the withdrawal of vitamin trace mineral mixture from broiler finisher diet supplemented with phytase on feed cost 1

Diet	Cost of formulation (Rs)	Feed cost/Kg empty carcass	Feed cost/Kg Saleable carcass
Ph+VTM+	75	150.9	127.2
Ph–VTM+(control)	74.6	148.3	126.5
Ph+VTM–	72.5	146.5	119.5
Ph–VTM–	72.1	157.5	132.5

1. As of Oct 2012

References

Atapattu, N.S.B.M. and Lal, P.K. (2009) Effects of Feed Withdrawal on Growth Performance and Carcass Parameters of Broiler Chicken. *Sri Lanka Journal of Animal Production*. 5(1), 1-14

Boa–Amponsem, K. Dunnington, E.A. and Siegel, P.B. (1991) Genotype, Feeding Regimen, and Diet Interactions in Meat Chickens. 1. Growth, Organ Size, and Feed Utilization. *Poultry Science*, pp. 70, pp. 682 – 688.

Deyhim, F. and Teeter. R. G. (1993) Dietary Vitamin and/or Trace Mineral Premix Effects on Performance, Humoral Mediated immunity, and Carcass Composition of Broilers During Thermoneutral and High Ambient Temperature Distress. *Journal of Applied Poultry Research*. 2, pp. 347–355

INAL, F. COŞKUN, B. GÜLŞEN, N. and KURTOĞLU, V. (2001) The Effects of Withdrawal of Vitamin and Trace Mineral Supplements from Layer Diets on Egg Yield and Trace Mineral Composition. *British poultry science*, 42(1), pp. 77-80.

KHAJALI, F. KHOSHOEI, E. A.and MOGHADDAM, A. Z. (2006) Effect of Vitamin and Trace Mineral Withdrawal from Finisher Diets on Growth Performance and Immunocompetence of Broiler Chickens. *British poultry science*, 47(02), pp. 159-162.

MAIORKA, A. LAURENTIZ, A. C. SANTIN, E. ARAUJO, L. F. and MACARI, M. (2002) Dietary Vitamin Or Mineral Mix Removal During the Finisher Period on Broiler Chicken Performance. The *Journal of Applied Poultry Research*, 11(2), pp.121-126.

Palo, P.E., Sell, J.L. and et al. (1995. Effect of early nutrient restriction on broiler chickens.2.Performance and digestive enzyme activities. *Poultry Science*, 74: 1470 – 1483.

ROSEBROUGH, R.W. STEELE, N.C. McMURTRY, J.P. and PLAVNIK, I. (1986) Effect of Early Feed Restriction in Broilers. II. Lipid Metabolism. *Growth*, 50, 217 – 227.

SAYADI, A. J. NAVIDSHAD, B. ABOLGHASEMI, A. ROYAN, M.and and SEIGHALANI, R. (2005) Effects of Dietary Mineral Premix Reduction or Withdrawal on Broilers Performance. International *Journal of Poultry Science*, 4(11), pp. 896-899.

SHAHRASB, M.A. MORAVEJ, H.and SHEILA M.S. (2012). Comparison of Different Levels of Vitamin Premix on Meat Lipid Oxidation in Floor and Battery Cage Broiler Raising Systems. *Journal of Advanced Veterinary Research*, 2, pp. 91-98

SIAHPOUR, S. TORSHIZI, M. A. K. SHARIATMADARI, F. and NIKNAFS, F. (2010) Effect of Vitamin And Mineral Premixes Withdrawal Time on Growth and Economic Performance of Broiler Chickens. *Journal of Veterinary Research*, 65(1), pp. 13-18.

SKINNER, J. T. WALDROUP, A.L. and WALDROUP. P. W. (1992) Effects Of Removal Vitamin and Trace Mineral Supplements from Grower and Finisher Diets On Live Performance and Carcass Composition of Broilers. *Journal of Applied Poultry Research*, 1, pp.80–286.

VIVEROS, A. BRENES, A. ARIJA, I. and CENTENO, C. (2002) Effects of Microbial Phytase Supplementation on Mineral Utilization and Serum Enzyme Activities in Broiler Chicks Fed Different Levels of Phosphorus. *Poultry Science*, 81(8), pp.1172-1183.

WANG, Z. CERRATE, S. YAN, F. SACAKLI, P. and WALDROUP, P. W. (2008) Comparison of Different Concentrations of Inorganic Trace Minerals in Broiler Diets on Live Performance and Mineral Excretion. *International Journal of Poultry Science*, 7(7), pp. 625-629.

WOYENGO, T. A.and NYACHOTI, C. M. (2011). Review: Supplementation of Phytase and Carbohydrases to Diets for Poultry. *Canadian Journal of Animal Science*, 91(2), pp. 177-192.

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Life cycle of the cotton mealybug *Phenacoccus solenopsis* in shoe flower plants under the Laboratory conditions

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Abstract: The cotton mealybug *Phenacoccus solenopsis* Tinsley (1989) is one of the invasive species recently introduced to Sri Lanka and nowadays it is wide spread among various parts of the country. The life cycle of *P.solenopsis* was studied under the laboratory conditions using *Hibiscus rosa-sinenesis* (Shoeflower) as host plant. This paper describes the lifecycle and discusses about the reproductive parameters of *P.solenopsis* under laboratory conditions relative to the appearance of symptoms on the host plant and the importance of making management interventions during the effective reproductive period of the insect.

Keywords: *Hibiscus rosa-sinenesis,* Neonate crawlers, *Phenacoccus solenopsis,* Reproductive period.

Introduction

The mealybug *Phenacoccus solenopsis* Tinsley (Hemiptera: Pseudococcidae) has a wide geographical distribution with its origin in Central America (Fuchs et al., 1991;Williams and Granara de Willink, 1992) followed by reports of the Caribbean and Ecuador (Ben-Dov,1994), Chile (Larrain, 2002), Argentina (Granara de Willink, 2003), Brazil (Mark and Gullan, 2005). *P.solenopsis* has been described as a serious and invasive pest of cotton in Pakistan and India (Hodgson *et al*., 2008) and on *Hibiscus rosa-sinensis* in Nigeria (Akintola and Ande, 2008). Latest report on the invasiveness of *P. solenopsis* has been from the Eastern region of Sri Lanka (Prishanthini and Vinobaba, 2009) on ornamentals, vegetable crops, and weeds, and in China (Wang et al. 2009; Wu and Zhang, 2009) on shoe flower. A detailed comparative study of few species of *Phenacoccus* including the Indian and Pakistan species, and details on the existence of seasonal morphological variations in *P.solenopsis* were provided by Hodgson *et al*. (2008).

Being a polyphagous pest, the P. solenopsis has been recorded to feed on a number of cultivated crops including weeds (Patel et al. 2009). According to the recent information provided by the authors (Prishanthini and Vinobaba, 2011) P.solenopsis has been reported from 28 host plant species comprising 10 families in Sri Lanka. In spite of its occurrence as a pest of several agricultural and horticultural crops since last few years, the information on its biology was scanty. Therefore, the present study on life cycle of *P. solenopsis* was carried out in the laboratory, so that the information generated may be used to formulate the management strategy of the pest.

Materials and Methods

Collection of insects

Studies on biology of *P.solenopsis* were conducted at the Zoology laboratory of Eastern University Sri Lanka using the population collected from unsprayed *Hibiscus rosa-sinenesis* plants in home gardens. Mealybug specimens of mealybugs used for the study were confirmed as *P.solenopsis* by the second author.

Rearing of insects

To establish initial culture of *P. solenopsis*, twigs of the host plants infested with adult females were brought to the laboratory individuals were separated and inoculated on shoe flower plants planted in the pots and reared in the laboratory. After about three days, the female mealy bugs settled on host leaves and stems and started egg laying. The crawlers emerged out and started feeding on the shoe flower plant.

The newly hatched crawlers were placed on shoe flower leaves with the help of fine camel hair brush. For that individual leaves with petioles of same size and maturity were collected from the shoe flower plants which did not exposed to any previous pesticide applications and free from mealybug infestation, were washed with tap water, shade dried and used as food source. The leaf petiole of the shoe flower leaves were wrapped with cotton wool dipped in water to keep the leaves turgid. Each leaf was infested with an adult female mealybug individual and was individually transferred to separate glass Petri plates (15 X 2 cm) each containing a shoe flower leaf. The study was conducted between May and June 2011 in the laboratory when maximum and minimum temperature and mean relative humidity of the study area ranged from 31.8 to 37.8 ° C and 23.8 to 27.5° C, and 53 to 81 % RH respectively.

Data Collection

When the newly emerged crawlers settled for feeding on shoe flower leaves, the crawlers were marked by drawing a circle around them. The crawlers thus marked were observed daily in the morning till they attained adult stage for further aspects of biology.The eggs laid by females of *P. solenopsis* were examined under binocular microscope for colour, shape and size. The adult female of mealy bug were picked up and placed individually on shoe flower leaf with the help of fine camel hair brush. The leaves were kept turgid for longer period as described earlier. The individual leaf was kept in glass petridish and observed daily under microscope till egg laying.

The time of egg laying was noted. Freshly laid eggs were counted and transferredto fresh shoe flower leaves. Time taken for egg hatching was recorded to obtain the incubation period. Hatching percentage of eggs was calculated from the number of eggs hatched out of total number of eggs kept under observation. The freshly emerged nymphs were marked individually on shoe flower leaves and observed daily under microscope to note moulting process The moulting was confirmed by the presence of exuivum on the leaf or on the posterior end of nymphs. The colour, shape and size of each nymphal instar were critically observed.

Adult females emerged after the last moult was observed for colour and shape. Measurements of the females were made by using measuring scale. Similarly, adult males emerged out from the silken cocoons were observed under microscope to study their colour, shape and size. Freshly emerged females were reared separately on shoe flower leaves to study their pre-oviposition, oviposition and post-oviposition periods. Since the female laid their eggs in shoe flowery sac located at posterior end of its abdomen, the ovisacs were collected during the oviposition period and counted the number of eggs in each ovisac for calculating fecundity. Longevity of male and female was assessed separately i.e. days of survival from emergence to the death of adults. Total 150 newly hatched crawlers were reared on shoe flower leaves up to third instar to determine the sex ratio. The third instar stage forming cocoons were separated as male and female and sex ratio was worked out. Total life cycle of female and male was calculated from the egg laying to the death of adult stage.

Data Analysis

Data were statistically analysed using statistical software Minitab 15.0

Results and Discussion

To understand the mode and degree of its population growth of an insect pest, it is important to understand the environmental conditions of the crop. Since a study of the life history and pattern of biological activities are difficult under field conditions because of the interference of biotic and abiotic factors, laboratory studies have become essential. Studies conducted in the laboratory using shoe flower leaves placed in Petri plates with detailed observations of reproductive and developmental stages of *P. solenopsis* formed the basis for the present study. Shoe flower leaves collected from the same position on the plant provided a similar food source for developing mealybugs thus avoiding any variation in food quality. Since individual leaves could be placed in Petri plates, they were easily observed under the microscope.

The *P. solenopsis* female laid their eggs in cottony ovisac located at posterior part of abdomen. The eggs

were smooth translucent, light creamy yellow in colour and oblong in shape with tapering ends (Fig 2). *P. solenopsis* exhibited variation in males and females at immature stages itself. The female nymphs moulted three times and males four times. Freshly emerged first instar nymphs were oblong in shape, dorsally convex, light yellow in colour with three pairs of legs and a pair of seven segmented filiform antennae. Body colour of newly hatched nymphs changed to pale white within two days after hatching from eggs. The newly emerged nymphs (Fig 2) crawled over to leaf surface for some time in search of suitable place for feeding and then settled down.

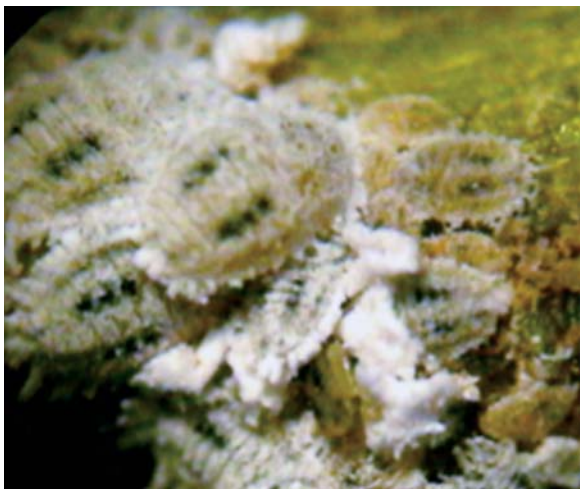

Figure1: Colony of adult female of *P.solenopsis*

Duration of first instar nymphs lasted for 4 to 6 days with an average of 3.24 ± 2.11 days (Table 1). After first moult, the second instar nymphs found to be oblong and yellow in colour. The second instar nymphs were similar to that of first instar nymphs in general appearance and morphological features, except in size. The antennae showed a marked increase in size but remained seven segmented. They secreted white waxy powder and waxy fibres on dorsal side after about 24 hours of first moult. The exuvium of the instar was seen near the posterior end of the abdomen. Duration of second instar nymphs ranged from 3 to 7 days with an average of 4.75 ± 3.28 days (Tab 1). Male and females of *P. solenopsis* nymphs can be distinguished from third instar onwards. The male nymphs formed a white silken cocoon after their third moult, but no such phenomenon in females. They continued to moult for remain in juvenile stage still. Male cocoons were cylindrical in shape and white in colour. Duration of male lasted for 5 to 7 days with an average of 6.06 ± 4.03 days (Tab 1).

Third instar nymphs of females were oblong in shape with yellow in colour (Fig 2). There were two pairs of dark black coloured spots with number of prominent glassy fibres of wax on dorsal surface of its body. Female bears a pair of prominent compound eyes, a pair of 7 segmented filiform antennae and three pairs of well-developed thoracic legs.

Duration of third instar nymphs ranged from 4 to 6 days with an average of 5.20 ± 0.45 days (Tab1). Adult males of *P. solenopsis* (Fig 2)were delicate, slender and elongated in shape. The colour of head, thorax, antennae and legs was yellowish-brown, whereas abdominal region pale yellow. A pair of well-developed metathoracic milky white wings and three pairs of well-developed legs could be seen easily. The antennae were ten segmented and found to be much longer than that of female antennae. It was as long as the total body length of males. Two pairs of waxy filaments were present at anal end of body of which the inner pair was long while the outer pair was short or to an extend half of the inner pair. Longevity of males ranged from 1 to 2 days with an average of 1.5±0.5 days and total life cycle ranged from 23 to 30 days with an average of 27.41 ± 1.10 days (Tab 1)

Female adults of *P. solenopsis* were oblong in shape and light to dark in colour having two pairs of black spots/strips on dorsal side of body region. Females were apterous, soft bodied, well distinguished segmented and body covered with white dusty secretion. It also possessed a pair of brownish, short, eight segmented filiform antennae and three pairs of red coloured legs. Longevity of female ranged from 32-55 days with an average of 34.3 ± 2.64 days. Total life cycle lasted for 55 to 60 days with an average of 58.3 ± 2.64 days (Tab 1). Observations on preoviposition, oviposition, and post oviposition periods of *P. solenopsis* revealed that it varied from 2 to 8, 12 to 18 and 7 to 9 days with an average of 8.56 ± 0.61, 16.73 ± 0.57 and 9.33 ± 0.47 days, respectively (Tab 1). Total

number of eggs laid by a single female during its entire life period ranged from 212 to 772 eggs with an average of 574 ± 82 eggs. The sex ratio of *P. solenopsis* in laboratory culture revealed that out of 330 third instar nymphs, 272 were females and 58 were males. Thus female to male ratio was 1: 0.21. The present study is first report on detailed reproductive biology of *P. solenopsis* from Sri Lanka. However, majority of observations match with the biological features of *P. solenopsis* on *Hibiscus rosa-sinenesis*as explained by Akintola and Ande (2008) from Nigeria and with the observations of Vennila *et al* (2010) in cotton plants in India. Shoe flower and cotton and some of the other preferred hosts which are agricultural crops are of Family Malvaceae. Therefore the results would be comparable to other crops and weeds act as host of *P.solenopsis*. The present study would lead a better understanding of incidence and spread of mealybug, *P. solenopsis* in shoe flower and alternate hosts which may be used in drafting management strategies. Lower numbers and shorter life span of males suggested that they have a minor role in reproduction, although under field conditions sexual reproduction also could be a possibility. In relation with the biology of *P.solenopsis* it is quite clear that the longevity of the adults, and their larger size with increased waxy coating, and higher food requirement, result in visibility of the pest and symptoms on the crop. Therefore, with the initial notice of *P. solenopsis* infestation on few plants it is essential to monitor the plants regularly for at least 14 to 20 days, which is when reproduction by females occurs, to make management decisions for using insecticidal sprays. Higher mortality of the crawlers, the longer effective reproductive period and increased longevity of adult females along with the expected natural mortality factors such as predation, parasitization and action of abiotic factors on crawlers and adults under natural field conditions, suggest that management interventions should be focused against reproducing adult females rather than crawlers to prevent the multiplication and spread of the pest. Therefore bioassay studies should use adult females instead of crawlers to determine an efficacious management scheme.

Table 1:
Lifecycle and reproductive parameters of *P.solenopsis* reared in shoe flower plants

Stage	n	Duration (days)	
		Range	Mean± S.D
Eggs	50	32-75	48.34 ± 5.67
Nymph			
1st instar	25	4-6	3.24 ± 2.11
2nd instar	25	3-7	4.75 ± 3.28
3rd instar	25	4-6	5.20 ± 0.45
Cocoon (Male)	25	5-7	6.06 ± 4.03
Adult			
Male	25	10-17	14.52 ± 2.92
Female	25	12-21	16.32 ± 7.76
Female			
Pre oviposition	25	2-8	4.88 ± 1.22
Oviposition	25	12-18	15.56 ± 3.42
Post oviposition	25	7-9	8.01 ± 2.18
Total life cycle			
Male	25	23-30	27.41 ± 1.10
Female	25	55-61	58.3 ± 2.64
Male after maturity	25	1-2	1.5 ± 0.10
Fecundity	25	212-772	574.4 ± 82.0

n- Number of observations

Longer developmental duration of males compared to females was due to an additional moulting and prepupal processes.. While the longer developmental period of the 2nd instar of males along with their high mobility could be the reason for their lower survival, it was not observed in the fourth instar due to the scarce population of males, together with the difficulty of *observation of any sex related differences during early* crawler stages.Akintola and Ande (2008) studied *P. solenopsis* on *H. rosa-sinensis* and found progressively increasing developmental periods of 6, 8 and 10 days for the 1st, 2nd and 3rd instars, respectively.

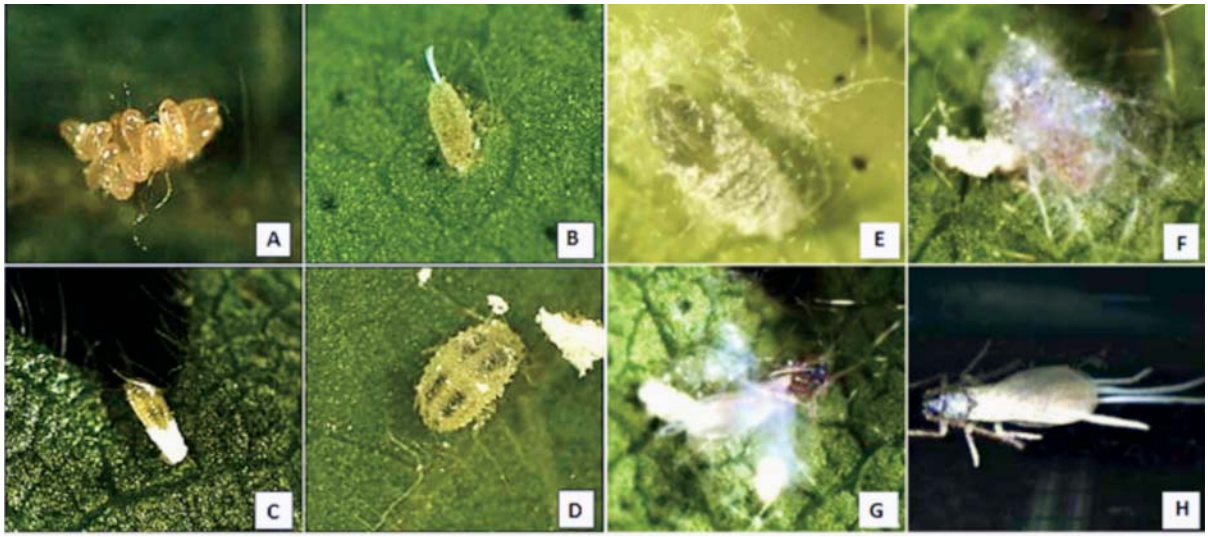

Figure 2:
Female life stages : A. Egg, B. First instar, C. Second instar D. Third instar and Male life stages: E. Third moult, F. Fourth moult, G. Adult ready for emergence H. Adult.

However *P. solenopsis* under laboratory conditions had longer developmental periods for the 2nd instar over the other two instars, indicating the influence of ecological zone with the associated weather conditions as well as host plants that could influence *P.solenopsis* development. The total developmental duration of a closely related species *Phenacoccus madeirensis* reared under constant temperatures of 25, 20 and 15^0 C was reported to be 30, 46 and 66 days respectively (Chong et al. 2003). This suggested that *P. solenopsis* has become acclimatized to a tropical environment that may have allowed its rapid spread across widely differing agro climatic zones of the country.

Further studies are required to determine developmental rates at different constant temperatures in growth chambers, ability of *P. solenopsis* to multiply, survive and spread across regions among many host plants, and the continuing molecular studies on the variations in their populations would be able to resolve and strengthen the species identity, biology and effect of environmental factors.

References

Akintola AJ, Ande AT. 2008. First Record of Phenacoccus solenopsis Tinsley (Hemiptera: Pseudococcidae) on Hibiscus rosa-sinensis in Nigeria. Agricultural Journal (Medwell Journals, Pakistan) 3(1): 1-3.

Ben-Dov Y. 1994 A systematic catalogue of the mealybugs of the world, p.686. Intercept Limited, Andover, UK.

Chong JH, Oetting RD, Iersel MW Van. 2003. Temperature effects on the development, survival and reproduction of the Madeira Mealybug, Phenacoccus madeirensis Green (Hemiptera: Pseudococcidae), on Chrysanthemum. Annals of Entomological Society of America 96 (4): 539-543.

Fuchs TW, Stewart JW, Minzenmayer R, Rose M. 1991. First record of Phenacoccus solenopsis Tinsley in cultivated shoe flower in the United States. South western Entomologist 16(3): 215-221.

Hodgson CJ, Abbas G, Arif MJ, Saeed S, Karar H. 2008. Phenacoccus solenopsis Tinsley (Sternorrhyncha: Coccoidea: Pseudococcidae), an invasive mealybug damaging shoe flower in Pakistan and India, with a discussion on seasonal morphological variation. Zootaxa 1913: 1-35.

Larrain SP. 2002. Insect and mite pes incidence on sweet pepinos Solanum muricatum (Ait.) cultivated in the IV Region, Chile. Agricultura-Tecnica 62(1): 15-26.

Mark PC, Gullan PJ. 2005. Anew pest of tomato and other records of mealybugs (Hemiptera: Pseudococcidae) from Espirito Santo, Brazil. Zootaxa 964: 1-8.

Patel HP, Patel AD. and Bhatt NA. 2009, Record of coccinellids predating on mealy bug, Phenacoccus solenopsis Tinsley in Gujarat. Insect Environ. 14(4): 179. http:/www.ncipm.org.in.

Prishanthini M. and Vinobaba M. 2009 ,First record of new exotic Mealybug species, Phenacoccus solenopsis Tinsley (Hemiptera: Pseudococcidae), its Host range and abundance in the Eastern Sri Lanka, Journal of Science 6(1),88-100

Prishanthini M and Vinobaba M.2011, A survey of mealybugs and their natural enemies in home gardens of Batticaloa, Sri Lanka, Proceedings of 10th Annual Research Session.2011,Eastern university, Sri Lanka, 1-2 December 2011, 202-208

Vennila S, Deshmukh AJ, Pinjarkar D, Agarwal M, Ramamurthy VV, Joshi S, Kranthi KR, Bambawale OM. 2010. Biology of the mealybug, Phenacoccus solenopsis on cotton in the laboratory. Journal of Insect Science 10:115 available online: insectscience.org/10.115

Williams DJ, Granara de Willink MC. 1992. Mealybugs of Central and South America, p.635. CAB International.

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Application of Biotechnology to Boost Economy: A Review with Special Reference to Sri Lanka

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Abstract: Biotechnology is the multidisciplinary integration of sciences and engineering in order to utilize the huge biochemical potential of biological systems for the protection, restoration and preservation of the environment and for the sustainable use of natural resources. The diverse disciplines of biotechnology reinvigorated by advances in emerging genomics, proteomics and metabolomics. The developments of biotechnological and engineering techniques are applied to solve many environmental, agricultural and industrial problems. In addition, agricultural biotechnology has been advancing very rapidly among the major disciplines of biotechnology. Although, it would give many promises in Sri Lankan contexts, it also poses as many questions.

Although, biotechnology has been at the forefront of scientific enterprise during the last three decades or more, Sri Lanka has failed to make progress in the field. Lack of human resources and sophisticated laboratory facilities may have greatly retarded advancement in this area. Therefore, this review will address the current status of application of biotechnology in the world and its present position in Sri Lanka.

Keywords: Biotechnology, applications, biotech products, research policy

Introduction

Sustainable economic growth is one of the key strategic challenges for the 21st century. Innovative technological change has raised livings standards, improved quality of life and enabled mankind to combat hunger, disease and environmental degradation. The expansion of biotechnology in a growing number of economic sectors has played an important role in contributing to this change and has enormous potential to improve a broad range of human activities (Ernst and Young, 2011).

As this technology continues to develop and rapidly change a wide range of activities from the delivery of vaccines to manufacturing processes, growing numbers of governments and international organisations have increased their attention on how to stimulate its development. The importance of biotechnology was realized all over the world within the last three decades. In many countries governments as well as private sector organizations have given high priority for R & D in biotechnology. As such, commercialization of biotechnology has become a major industry worldwide (Table 1. and fig. 1)

Traditional biotechnology involves mainly fermentation technology i.e., manufacturing of bread, alcohol, wine, beer and fermented milk products using microorganisms. The modern biotechnology includes recombinant DNA technology, tissue culture and mutagenesis.

Rank 2012	Company	Country	Market Cap in 2012 (USD billions)	Market Cap in 2011 (USD billions)	Website
1	Novo Nordisk	Denmark	76.92	60.09	novonordisk.com
2	Amgen	USA	60.09	49.72	amgen.com
3	Gilead Sciences	USA	40.16	32.68	gilead.com
4	Biogen Idec	USA	34.26	25.6	biogenidec.com
5	Teva Pharmaceutical Industries	Israel	34.23	42.96	tevapharm.com
6	Baxter International	USA	32.27	33.36	baxter.com
7	Celgene	USA	28.38	27.52	celgene.com
8	Merck KGaA	Germany	21.16	20.14	merckgroup.com
9	CSL	Australia	21.11	18.33	csl.com.au
10	Alexion Pharmaceuticals	USA	18.85	10.48	alxn.com

Source: FAO report, 2012

Methods and Materials used in Biotechnology

Advances in biotechnology have always been based on the development of new methods. In traditional processes developed centuries ago, such as the production of wine, bread and various fomented foods. These methods were developed over long periods of experience, in an essentially 'trial and error' process with the aim to 'conserve and reproduce successful recipes'.

Modern Biotechnology brings together subjects such as microbiology, engineering, agriculture, genetics and biochemistry, in a combined effort to increase production of commercially interesting products or to solve environmental problems. Various methods are employed at the different steps of a biotechnology process: upstream processing (inoculums and substrate preparation), bioconversion (by microorganisms, plants or animals or parts thereof), and downstream processing (product recovery, waste disposal) (David and Adrina, 2002).

The Biotechnology Industry

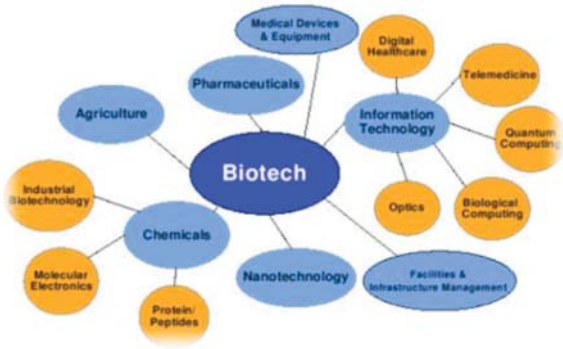

Fig. 1:
Biotechnology industries in the world.

Biotechnology Applications

Biotechnology has benefits to society in the health, environmental, agricultural and food sectors (Table 2). In the pipeline, there are products that can help address famine and malnutrition, improve human health and reduce the environmental impact of industrial activities. Biotechnology provides an increasing range of tools for industry to improve economic and environmental performance beyond what could normally be achieved using conventional technologies.

Table 2:
Common Applications of Biotechnology

Agriculture Improved foods, pest control, plant and animal disease control, improved food production. Stress tolerance (fig.2).	**Industry** Oil/mineral recovery, environmental protection, waste reduction. Improved detergents, chemicals, stronger textiles.
Health Care Drugs, vaccines, gene therapy, tissue replacements.	**Research** Understanding the human genome and better detection of diseases.

Experts on the World Economic Forum's Council on Biotechnology have selected the following 10 developments which they believe could help not only meet the rapidly growing demand for energy, food and healthcare, but also increase productivity and create new jobs, should issues such as regulatory certainty, public perception and investment be tackled successfully (John and Henry, 2006).

1. Bioproduction of sustainable chemicals, energy and other materials
2. Genetically modified crops to increase sustainable food production
3. Seawater bioprocesses to produce fuel and chemicals
4. Zero-waste bio-processing
5. Carbon dioxide as a raw material
6. Regenerative medicine to create new organs
7. Rapid and precise development and manufacturing of medicine and vaccines
8. Accurate, fast, cheap, and personalized diagnostics and prognostics
9. Biotech improvements to soil and water
10. Advanced healthcare through genome sequencing

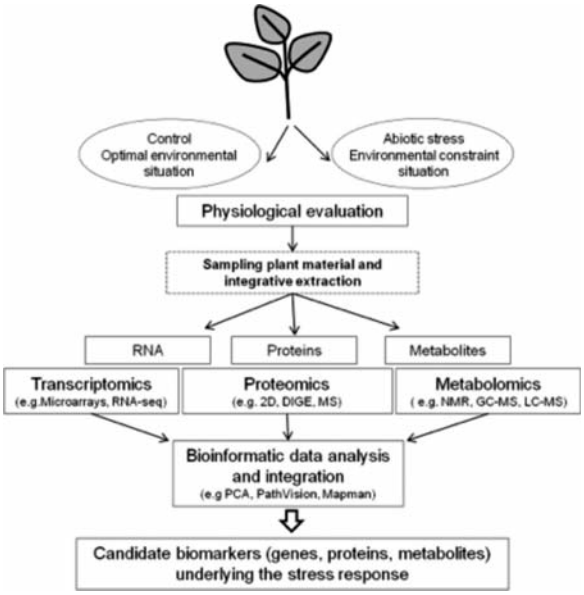

Fig. 2: Schematic overview of a common System Biology approach to study abiotic stress responses in plants.

Where does Sri Lanka stand in the development of biotechnology industries?

Although biotechnology has been at the forefront of scientific endeavor during the last three decades or more, Sri Lanka has failed to make headway in this field. Lack of trained personnel in the different areas of biotechnology as well as the lack of sophisticated laboratory facilities may have greatly related advancement in this area in Sri Lanka.

In spite of these deficiencies, there are few biotech industries involved in tissue culture, fermentation, diagnostics and bioremediation. There is a great potential for agricultural biotechnology in Sri Lanka as a tool in crop improvement programmes. Future developments in other fields like medicine, natural resources, industry, and food science waste management, etc. will be possible on advances made through novel biotechnological approaches.

Some private companies are involved in various agro biotech activities, more specifically in plant tissue culture. They have well developed tissue culture laboratories set up in 1980s. Some of the private companies, namely, Serendib Horticulture Technologies (Pvt.) Ltd., CIC Agro Industry, Hayleys Agro Biotech, Mike Flora (Pvt.) Ltd., and Ramya Horticulture are involved in micropropagation of ornamental plants, fruit crops, economically important plants and vegetables, exporting cut flowers, foliage, rooted plants, and accessories, production and sale of agricultural seeds, and production and conservation of aquatic plants. Many of the private tissue culture laboratories in the country are mainly involved with the production of ornamental plants for the local and/or the foreign market. Some companies such as Serendib Horticulture and Hayleys Agro Biotech do large volumes of fruit plants, especially Banana and Pineapple as well. In addition to the above, some companies are working as consultants and collaborators with foreign governments and companies. Very few companies have public-private partnerships within the country.

Sri Lanka does not have much progress in medical biotech industries. A few private laboratories are involved in molecular diagnostics, PCR techniques, DNA fingerprinting and genotyping. Recently some companies in the field of medicine started to develop diagnostic kits, paternity testing, etc. A few local biotech industries are involved in producing bio-fertilizers and biopesticides.

Alcohol fermentation, especially brewing beer is the most mature area biotechnology in Sri Lanka. There are many examples of domestic, small and medium scale industries applying these techniques.

Commercial application in biotechnology and the establishment of biotechnology industries need more state patronage to make it sustainable. The private sector in Sri Lanka is yet to play an important role in contributing to the economic development through biotechnology innovations. Although heavy investments in biotech industries may not be possible, with the available physical and human resources. Sri Lanka can exploit the full potentialities of biotechnology. A crucial issue is lack of proper mechanism for transferring mature technologies.

Advances in biotechnology have also led to address safety and ethical aspects of biotech industry and products. Quarantine regulations should be streamlined to facilitate research and innovation. Guidelines should be developed for collaborative research with foreign scientists/organizations involving local biological materials. Patenting is also very important in commercialization of products or processes.

Some of the potential Industrial applications of biotechnology for improving agriculture, human health and environment in Sri Lanka are:

- Development of recombinant vaccines for infectious diseases i.e., dengue, tuberculosis, and hepatitis
- Development of immuno diagnostics, drugs, antibiotics and steroids, monoclonal antibodies, human insulin, vitamins and amino acids, DNA probes, DNA fingerprinting, molecular testing services.
- Screening for stress factors in vitro and producing diseases free plants, crops with improved nutritional value.
- Development of fermentation methods and facilities for production of ethanol
- Production of enzymes, chemicals, organic acids, industrial solvents, flavours
- Enhancement of industries in biofertilizers, biopesticides, bioherbicides
- Production of biofuels, bioethanol, bio-gas
- Bioremediation and biofilteration

Why should biotechnology industries be promoted in Sri Lanka?

Sri Lanka is mainly depending on agriculture and agriculture based products to feed its population. As Sri Lanka has a high potential and scope for technological advances in biotechnology and commercialization of agricultural biotechnology the productivity of agriculture should be improved with the available technologies in order to achieve food sufficiency.

There is also growing demand for animal based foods in the developing countries, hence, this demand could be met by using technologies in modern biotechnology such as artificial insemination, embryo transfer, etc., Modern biotechnology increases overall productivity, nutritional status and resistance to diseases and extreme environmental conditions such as drought, salinity etc. it also helps to produce diagnostic tools and vacancies to control animal diseases. DNA Typing is now widely used in Sri Lanka for both criminal and civil procedures. DNA finger printing facilities are provided by Genetech, a private biotech company established in the country.

Future Prospects to develop Agricultural biotechnology industries in world

The future of biotech crops looks encouraging. A number of developing countries are expected to plant biotech crops before 2015, especially the Asian countries. Some African countries may also contribute in the biotech crop hectarage in the near future, with the first drought tolerant maize planned for release in Africa in 2017. The same biotech crop is expected to be released in North America in 2013; the first stacked soybean (with herbicide tolerance and insect resistance traits) will be planted in Brazil in 2013; vitamin-A enriched Golden Rice could be released in the Philippines in 2013 or 2014; drought tolerant sugarcane in Indonesia; and biotech maize in China. Biotech crops is not a panacea; but they have the potential to make a substantial contribution to the 2015 MDG goal of cutting poverty by half, by optimizing crop productivity, which can be achieved by public-private sector partnerships (OECD, 2011).

Future directions to be taken to promote biotechnology industry in Sri Lanka

1. Develop infrastructure, expertise and skills in biotechnology
2. Provide advanced training in already existing techniques uch as tissue culture, micro-propagation etc.
3. Strengthen the link between the private sectors and government research organizations in commercialization of research
4. Direct future development towards genetic improvement of crops, drought and disease tolerant varieties, improving nutritional quality of certain crops, production of useful hybrid varieties of plants
5. Attract joint venture partners from developed countries in order to access foreign markets for biotechnology products
6. Encourage biotech industry development through granting pioneering status
7. Promote and create public-private partnership programmes in research and product development in biotechnology
8. Setup a government body to approve locally developed testing kits (i.e., ELISA and PCR)
9. Protect Intellectual property rights and biodiversity
10. A biotech association should be formed with the biotechnologists in the public and private sectors: regular meetings to be conducted to discuss advancements and progress made, regional and international biotechnology network should be formed.

References

David A. Mitchell and Adriana contin, 2002. Methods in Biotechnology. 2:102-123.

Organization for economic co-operation and development (OECD): Key biotechnology indicators report, December 2011. Pp 1-4. France. .

Ernst and Young, 2011. Beyond Borders: Global biotechnology report. pp. 1-57.

John H. Vanston and Henry Elliott. 2006. Biotechnology: A guide to business and workforce development, Report of the technology futures Inc. . html

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Effect of simulated damage of Army worm, *Mythimna separata* (Walker) (Lepidoptera: Noctuidae) on yield compensation of irrigated rice

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Abstract: This simulated study was designed to investigate the relationship between army worm *Mythimna separata* (Walker) damage intensity and rice yield related traits at two different growth stages in irrigated rice. Army worm damage was simulated by making a cut at four different height levels from top to bottom above the soil surface at two growth stages under randomized complete block design with four replicates. The agronomic and reproductive traits were recorded. The results revealed that, the rice crop completely compensate the simulated army worm damage up to the 50% intensity at early growth stages but at late growth stages, it was progressively reduced. The damage intensity and time of damage had a significant effect on both agronomic and reproductive traits.

Keywords: Simulation, Rice compensation

Introduction

Rice (*Oryza. sativa* L.) is the world's most important crop and is the staple food for more than half of the population (Khush 2004). About 90% of rice production and consumption worldwide occurs in Asia (Gealy *et al.,* 2003). In Sri Lanka, rice is the most important crop occupying 34 percent (0.77 /million ha) of the total cultivated area. One of the major obstacles to rice production within the country is the lack of stable yields due to several major diseases, pests and weeds. Of the pests affecting rice production, *Mythimna separata* (Walker) (Lepidoptera: Noctuidae) is an occasionally serious pest, the larvae of which can defoliate complete crop in every growth stages.

Army worm, *M. separata* is a moth totally polyphagous which occasionally causes serious losses to rice crop. The adult moths are pale and brick red to pale brown and have a very hairy body covered with dark specks and patches. They are about 2-3 cm long and 3-5 cm wing spread. The adult moths are quiescent during the day. And moths feed on dew drops and start mating 1-3 day after emergence. The eggs are laid between the leaf sheaths and the stem near the joint of the leaf sheath and leaf blade. The average of 100 eggs in one cluster. The newly hatched larvae are 1.8mm long and 0.33 mm wide. The full mature larvae can cut off the rice panicles from the peduncle, the reason they are also called ear-cutting caterpillars. Gregarious nature of the larvae during the latter instars and their increasing feeding capacity are the apparent causes of their becoming suddenly noticeable. The insect hibernates as full grown larvae. The duration of hibernations and the number of generations in a year are controlled by temperature.

Traditionally farmers manage water level in the field to control the army worm and they believe that army worm damage in paddy fields bring them better harvest. Cutting tillers to simulate army worm damage on growing rice can also provide reasonable measures to prove this myth associated with damage intensity at particular growth stages of the rice crop. This study was designed to investigate the relationship between army worm damage intensity and rice yield related traits at two different growth stages. This relationship will be useful in developing economic models and better management strategies.

Materials and Methods

The experiment was conducted in an experimental garden at the Faculty of Agriculture, University of Ruhuna, Sri Lanka (longitude 6^0 3'21.07", latitude 80^0 33' 37.37"). For the field layout, complete randomized design was used with 4 replicates (plots) for each treatment. For each replicate (plot), 25 rice seedlings (14-day old), variety At 362 were planted in a 5m x 5m plot, with 25cm between hills and rows. All the management practices including fertilizer application, pest and disease management etc. were performed according to the recommendations of the Department of Agriculture, Sri Lanka. Weeds occurring in the plots were hand-removed periodically. The treatments were imposed to simulate army worm damage at five severity levels. 0% (control), 25%, 50% and 75% at two growth stages, (30 days after transplanting; at late seedling stage and 45 days after transplanting; just before booting). To avoid an edge effect, only the nine plants in the middle of the plot were characterized.

At the defined growth stages, tillers were cut at the defined heights from the base using scissors. In each plot, traits measured were; plant height (cm), number of tillers per plant, number of panicles per plant, panicle length (cm), 1000 grain weight (g) and number of seeds per panicle. The traits were measured by using the standard evaluation system for rice developed by the International Rice Research Institute (IRRI) as indicated in table1. Data were analyzed using two-way analysis of variance, using the factors of level of damage at 0%, 25%, 50%, 75% and cutting time at 4 weeks and 8 weeks after planting .The Duncan's Multiple Range Test (DMRT) were carried out to examine significant differences between factors.

Table 1:
Selected traits and their methods of measurements

Character	Method of measurement
Plant height (cm)	Height from ground level to the tip of the leaf of the tallest tiller
No of tillers	Total number of tillers per plant
No of panicles per plant	Total number of panicles per plant at maturity
Panicle length (cm)	Length measured from base to the tip of the panicle
1000-grain weight (g)	(Weight of 100 filled seeds/100)×1000
No of seeds per panicle	Average number of seeds from 3 panicles

Results

Our results revealed that the rice crop can completely compensate the damage caused by army worm by increased tillering and number of panicles up to 50% cutting intensity at early growth stage. The increment of cutting intensity at late growth stage caused significant reduction of both agronomic and yield related traits; Plant height, No of mature tillers per plant, No of panicles per plant, Panicle length, 1000 grain weight and No of seeds per panicle.

Damage compensation of Agronomic Traits

A significant variation was not found in plant height with cutting intensity at early stage ($P>0.05$).However, significantly shortest plants were observed in 75% intensity compared with others at late stage. Plant height ranged from 114 cm to 93.2 cm with an average of 105.52 cm (SE=3.88). Tiller number ranged from 12.6 to 8 with an average of 10.85 (SE=0.97).The significantly highest number of tillers (12.2) was observed at 50% cutting intensity .compared to that of 75% at early stage (30 days after transplanting). However, the tiller number was successively reduced with cutting intensity at the late growth stage (45 days after transplanting).There was no significant effect of cutting intensity up to 50% for the number of mature tillers at 30 days cutting time.

Damage compensation of yield components

There were significant differences ($p<0.05$) found in yield components with the cutting intensity at both stages comprising of the number of panicles, panicle length, thousand grain weight, and no of seeds per panicle(table 2). The significantly highest number of panicles (10.4) was observed at 50% cutting intensity while least number (7.53) was recorded at 75% intensity at 30 days after transplanting. Significantly

long panicles (27.31 cm), significantly higher no of seeds per panicle (179.02), and comparatively highest thousand grain weight (25.18g) were observed in 0% (control) compared to that of 75% cutting intensity at 30 days after transplanting. At late growth stage, all the reproductive traits were significantly highest in the control group (0%) compared to 75% cutting intensity and successively reduced with the increment of cutting intensity. Considering the overall effect of cutting intensity and cutting time, rice crop can compensate the damage up to 50% by increasing number of panicles per plant at 30 days after transplanting. In addition, progressive increment of losses were observed in relation to cutting intensity and cutting time for the panicle length, thousand grain weight and number of seeds per panicle.

The letters behind the mean value indicate significant differences between populations based on Duncan's Multiple Range Test. Mean with the same letters are not significantly different at $P<0.05$.

The response of the rice crop to simulated army worm damage

There were significant differences in the plant height, no of mature tillers at vegetative stage and no of panicles per plant, panicle length, and the number of seeds per panicle at reproductive stage among the time of damage, intensity of damage and the interaction of time and intensity of damage.

The cutting intensity and cutting time had a significant effect on both agronomic and reproductive traits, comprising of plant height, no of tillers, no of panicles per plant, panicle length, 1000 grain weight and no of seeds per panicle. Further, a significant interaction between timing and cutting intensity was observed on all traits except thousand grain weight (g).

Discussion

The yield loss was associated with damage intensity at the late stage of the simulated damage. Rice crop can completely compensate damage up to 50% cutting intensity by developing new tillers and reproducing mature panicles at 30 days after transplanting. At late stages, the damage compensation drastically reduced because of the insufficient time to produce mature tillers and harvestable grains by the time of crop maturity. Similar results have been reported with respect to various crops and pest damages (Brown 2005; Oyediran and Heinrichs 2002; Islam and Hossain 2003).

The compensation takes place raising the number of tillers and number of panicles at the low levels of cutting intensity of 25% and 50% at early growth stage (Table2) as damaged plants can produce more tillers than undamaged ones (Lv *et al.* 2008). However, the all agronomic and reproductive traits; plant height, number of mature tillers, number of panicles, panicle length, thousand grain weight, and total number of seeds per panicle were considerably reduced when damage occurred at high intensity during the latter growth stages (Table 02). Therefore, the time and intensity of damage had a crucial effect on the all agronomic and reproductive traits, as reported by Phung *et. al.* (2010). When damage occurred at later in the growth of the rice crop, there was no enough time for regenerating tillers and also to sufficiently mature at harvest, as explained by Oyediran and Heinrichs (2002) and Rubia *et al.* (1996). Re-tillering of rice crop occurred in that cutting tillers at all stages of growth but it needed sufficient time to produce mature tillers and panicles at harvest time.

Cutting tillers during early stages of the growth enabled to produce mature panicles at the harvest time. Yield loss was also found to be highest when damage was imposed at the latter growth stages. This result was supported by Haque *et al*. (1986) who had simulated rat damage in deep water rice in Bangladesh. However, no compensation was found in the current experiment for traits such as plant height, panicle length, thousand grain weight and total number of seeds per panicle. In addition, rice grain production is greatly affected by the number of mature tillers present at the flowering stage as only mature tillers can produce panicles (Phung *et. al,* 2010). Tillers formed late in the life of the plant will not bear panicles due to a lack of assimilates and some of them will die before reaching maturity (Chatterjee and Maiti, 1985) or will produce panicles which have empty husks when harvested

(Buckle *et al*., 1979). Therefore, tillers produced early in the growth stage of the rice plant will produce more yield than the late stage. (Awan *et al*., 2007).

In conclusion, the results revealed that, rice crop can totally compensate the simulated army worm damage up to the intensity of 50% damage at 30 days after transplanting, giving certain strength to the myth of the traditional farmers. Although indigenous methods are time tested, environmentally sound and have greater potential value for sustainable agriculture, those are inadequate to cope with the present pest problem since almost all farmers cultivate high yielding varieties which are vulnerable to pest attacks. Therefore, these findings have important implications for managing army worm damage in rice cultivation in Sri Lanka in terms of the timing and damage assessment.

References

Awan, T.H., Ahmad, M., Ali, I., Anwar, M., Manzoor, Z., (2007). Contribution of tillers within a rice plant to yield and yield components, *J. Agric. Res*, vol. 45, pp.237-243.

Brown, P.R., (2005). The effect of simulated house damage to wheat in Australia, *Crop Prot*.vol. 24, pp. 101-109.

Brown, E.S., Dewhurst, C.F., (1975). The genus *Spodoptera* in Africa and the Near East, *Bulletin of Entomological Research,* v*ol.65*, pp.221-262.

Tuckle, A.P, Yong, Y.C, Rowe, F.P., (1979). Yield response of the rice *Oryza-sativa* cultivar improved-Mahasuri to simulated rat Rattus argentiventer damage, *Malays. Agric. J.* vol. *52,* pp.135-144.

Chatterjee, B.N, Maiti, S (*eds.)* (1985). Principles and Practices of Rice Growing, *Oxford & IBH Publishing*, Calcutta.

Gealy, D.R, Mitten, D.H, Rutger, J.N., (2003). Gene flow between red rice (*Oryza sativa*) and herbicide-resistant rice (*O. sativa*): implications for weed management, *Weed Technol*,vol.17, pp.627–645.

Haque, M.E, Ahmed, M.S, Brooks, J.E., (1986). Effect of simulated rat damage on deep water rice in Bangladesh, *Bangladesh J. Agric*, vol. 11, pp.63-70.

Khush, G.S., (2004). Harnessing science and technology for sustainable rice-based production systems, *In: FAO Rice Conference*, pp.13.

Lv, J, Wilson, L.T, Longnecker, M.T., (2008). Tolerance and compensatory response of rice to sugarcane borer (Lepidoptera: Crambidae) injury, *Environ. Entomol*, vol. 37, pp. 796-807.

Oyediran, I.O, Heinrichs, E.A., (2002). Response of lowland rice plants to simulated insect defoliation in West Africa, *Int. J. Pest Manag*, vol. 48, pp. 219-224.

Phung, N.T.M, Brown, P.R, Luke, K.P.L, Tuan, L.M., (2010). The effect of simulated rat damage on irrigated rice yield and compensation, *Crop Protection,* vol.29, pp.1466-1471.

Rubia, E.G, Heong, K.L, Zalucki, M, Gonzales, B, Norton, G.A., (1996). Mechanisms of compensation of rice plants to yellow stem borer *Scirpophaga incertulas* (Walker) injury, *Crop Prot,* vol. 15, pp.335-340.

Table 02:
Mean comparison of selected traits with cutting intensity and cutting time

Cutting Time	Cutting Intensity	Traits					
		Plant height(cm)	No. of tillers/p	No. of panicles	Panicle length(cm)	1000 grain weight(g)	No of seeds/pa
30 days	0%	113.60[a] ± 3.47	10.67[a] ±1.75	9.40[a] ±1.25	27.31[a] ±0.9	25.18[a] ± 0.53	179.02[a] ± 11.70
	25%	114.20[a] ± 3.27	12.00[a] ±0.87	10.00[a] ±0.2	27.07[ab] ±0.5	24.76[a] ±0.27	170.73[ab] ± 1.1
	50%	110.47[a] ± 4.43	12.20[a] ±0.35	10.40[a] ±0.35	26.99[ab] ±0.9	24.74[a] ± 0.57	169.78[ab] ± 15.8
	75%	111.96[a] ±5.31	8.53[b] ± 0.5	7.53[b] ±0.3	24.85[b] ± 1.9	24.26[a] ± 1.02	141.56[b] ± 25.47
45 days	0%	111.80[a] ± 2.2	10.07[a] ± 0.12	8.67[a] ± 0.23	27.64[a] ± 0.33	24.94[a] ±0.98	187.67[a] ± 24.67
	25%	108.00[a] ±3.8	9.53[ab] ± 0.50	8.13[ab] ± 0.61	25.91[b] ± 0.21	23.26[ab] ± 0.67	163.47[b] ± 1.87
	50%	106.20[a] ± 4.54	9.47[b] ± 0.30	8.07[ab] ± 0.70	25.30[b] ± 0.51	23.06[ab] ± 0.66	153.13[b] ± 2.16
	75%	96.07[b] ± 3.82	9.10[b] ± 0.1	7.60[b] ± 0.35	20.49[c] ± 0.51	21.45[b] ± 1.68	83.40[c] ±2.83

Table 03:
Summary of two-way ANOVA for testing the effects of the cutting intensity (0%, 5%, 25% 75%), cutting time (30 days and 45 days after transplanting) and their interactions on the yield related traits.

Character	Cutting Intensity (I)			Cutting Time (T)			I*T		
	df	f	p	df	f	p	df	f	p
Plant height	3	5.57	*	1	18.99	**	3	3.64	*
No of tillers	3	9.35	**	1	18.03	**	3	6.49	*
No of panicles/ plant	3	10.16	**	1	24.9	**	3	4.98	*
Panicle length	3	34.3	***	1	23.15	**	3	7.49	*
1000-grain weight	3	6.13	*	1	18.24	**	3	2.1	NS
No of seeds/ panicle	3	26.92	***	1	9.74	*	3	5.87	*

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Effects of Different Explants and Hormones on Callus Growth and Regeneration of Radish (Raphanus Sativus L.) Var.Beeralu Rabu

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Abstract: This study was conducted to investigate an efficient *in vitro* propagation system for Radish (*Raphanus sativus* L. variety Beeralu Rabu). Six different hormone combinations on Murashige and Skoog's (MS) basal medium with 0.1 mg/l NAA + 0.1, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0 mg/l 2, 4 – D as well as three explant types: hypocotyl, leaves and root were employed. Complete Randomized Design (CRD) was used as experimental design with 5 replicates. Statistical analysis was carried out using the Student Newman-Kuells Means Separation Test of SAS program (9.1.3). After one month the callogenesis and diameter of fresh callus were evaluated. Calluses from selected explant were cultured to regenerate with 0.1 mg/l NAA and 1.0, 1.5, 2.0, 2.5, 3.0 mg/l BA. The results showed that, 0.1 mg/l NAA + 0.5 mg/l 2.4-D was the best medium for the callus induction and hypocotyl explant was the best explant in respect to the callogenesis and also, it had 4 callus with highest diameter (>2cm). These results are indicating the presence of the high internal hormonal concentration of the plants and probably it's inhibiting effects on callus production of the explants in high level hormone enriched media. Medium supplemented with 0.1mg/l NAA and 2.5mg/l BA was the best hormonal combination for the *in vitro* regeneration (6.6 shoots/explant) of Radish variety Beeralu from callus.

Keywords: *Raphanus sativus* L., Callogenesis, Regeneration, Explants, MS basal medium, Hormones

Introduction

Radish (*Raphanus sativus* L., 2n=18) is a common vegetable in Asia and in most parts of the world. This plant produces a red colored edible root with different shapes. Apart form culinary purposes; radish has diverse medicinal properties as well. Major genetic improvement of radish has been achieved by conventional plant breeding methods, such as crossing. However, these methods are time and labour consuming. In recent years, advances in plant genetic engineering have opened a new avenue for crop improvement and various transgenic plants with novel agronomic characteristics have been produced. The success in plant genetic engineering is dependent upon several factors, from which an efficient tissue culture system, with high plant regeneration potential, is a crucial option (Mohammad et al, 2009). It is generally well known that radish is one of the most recalcitrant crop plants in culture. Early studies focusing on plant regeneration via organogenesis from seedling explants, embryogenic calli and microspores revealed a shoot regeneration frequency too low for practical usage (Curtis, 2003). However, to the best of our knowledge, there is limited information on the plant regeneration from cell and tissue cultures of radish in Sri Lankan variety. The aim of this research was to study the effects of explant types and different hormone levels on the callus production and regeneration of Radish (Raphanus sativus L) variety Beeralu rabu.

Materials and Methods

Plant source

Seeds of Radish were purchased from the Seed and Planting Material Division, Department of Agriculture, Sri Lanka.

Establishment of aseptic cultures

Seeds were surface-sterilized by washing tap water, soapy water, immersing in 70% ethanol for 3 minutes, three times from distilled water and soaking in a 20% Clorox for 20 minutes respectively. Sterilized seeds were then rinsed three times in sterilized distilled water and inoculated on a medium comprised of half-strength MS salts, 3% sucrose and the medium was solidified with 0.3% Agar prior to autoclaving. The seeds were cultured under light for 10 days (Dahanayake *et al*, 2010).

Preparation of culture media

All the media used were adjusted to a pH value of 5.8 - 6.0 with 1N NaOH or 1N HCl solution, gelled with 0.3% agar (except those for seed germination and growth) prior to autoclaving at 1.4kgcm-2 for 20 minutes.

Callus production ability of different type of explant in different mediums

Leaf, hypocotyl and root explants of aseptic plantlets were cultured on MS basal medium. Six different media were used, where supplemented with 0.1 mg/l NAA and 0.1, 0.5, 1.0, 1.5, 2.0, 2.5, 3.0 mg/l 2, 4 – D to investigate the callus production ability with the 5 days old plant from seed germination. In cultures, leaves were cut into sections (0.5 cm2) and placed on media with the adaxial surface toward the media, while hypocotyl and roots were cut into about 5 mm and cultured by laying randomly on the media. Five replicates were used from each explant and cultures were kept under light.

Regeneration ability of callus from selected explant on different mediums

Callus induced from the hypocotyls were shifted to different regeneration media treated with 0.1 mg/l NAA and 1.0, 1.5, 2.0, 2.5, 3.0 mg/l BA after slicing 0.5cm parts and cultures were kept under light.

Data collection and analysis

Experiment was arranged according to the Complete Randomized Design (CRD). Callus induction and regeneration were evaluated 30 days after initiation. Number of explants with callus in different treatment and diameters of those calluses were recorded. Numbers of shoots regenerated from selected explant in regeneration media were recorded. All experiments had five replicates, each with five explants per bottle. Statistical analysis was carried out using the Student Newman-Kuells Means Sepa-ration Test of SAS program (9.1.3).

Result and Discussion

Establishment of aseptic cultures

After surface sterilization aseptic seedlings and explants were got from those aseptic plants (Fig.1a & b).

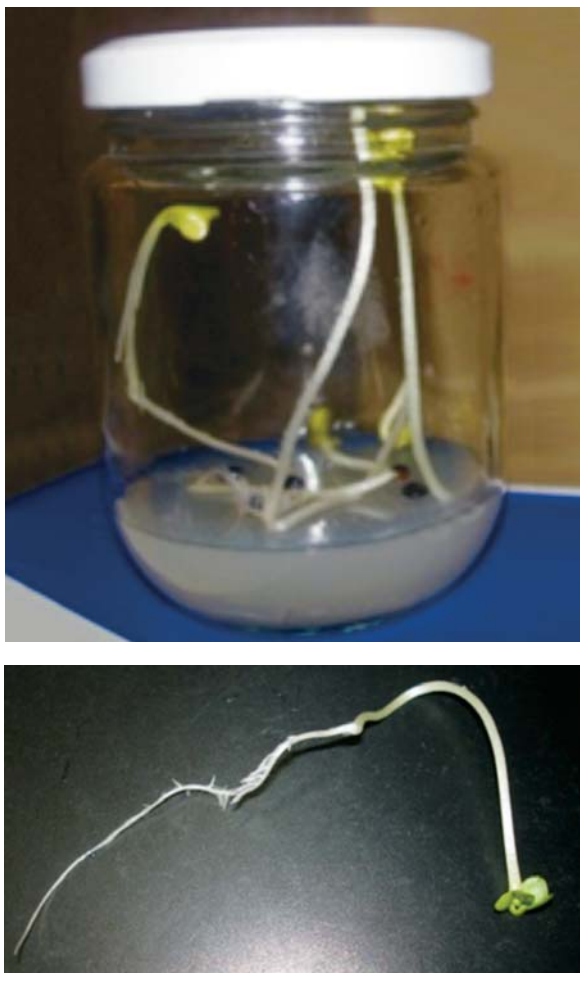

Figure 1. Aseptic seedlings and explants

Callus production ability of different type of explant in different treatments

Type of explant influenced directly for callus production mentioned in Fig.2

Hypocotyl explant was the best explants in respect to the callogenesis and also, it 4 callus diameter (>2cm) (Fig.3). Callus from hypocotyl explant was observed from every treatment except treatment 6.

Among all explants root explants had a least callogenisis ability in comparison with other explants (Fig. 4) and leaves were showed medium callogenesis ability among other explants (Fig.5).

The callus growth was remarkably affected by hormone. The treatment 2 (0.5 mg/l 2, 4-D and 0.1 mg/l NAA) showed highest callus formation as Fig.3, Fig.4 and Fig.5 in all explants.

It was low hormone concentration when considering other used hormone concentration in treatment 3, 4, 5 and 6. According Sachiko et al, 1990 callus growth is prohibited in hormone enriched media.

Root explant gave callus only in treatment 2 (Fig.4). The calluses were hard in texture and yellowish, greenish, brownish in color. First callus was immerged 6 days after culture day.

Fig.3 Callus from hypocotyl explants in 0.5 mg/l 2, 4-D and 0.1 mg/l NAA

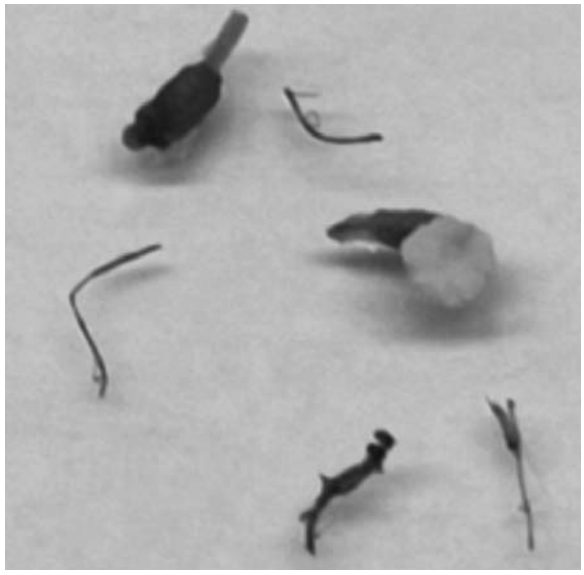

Fig.4 Callus from roots explants in 0.5 mg/l 2, 4-D and 0.1 mg/l NA

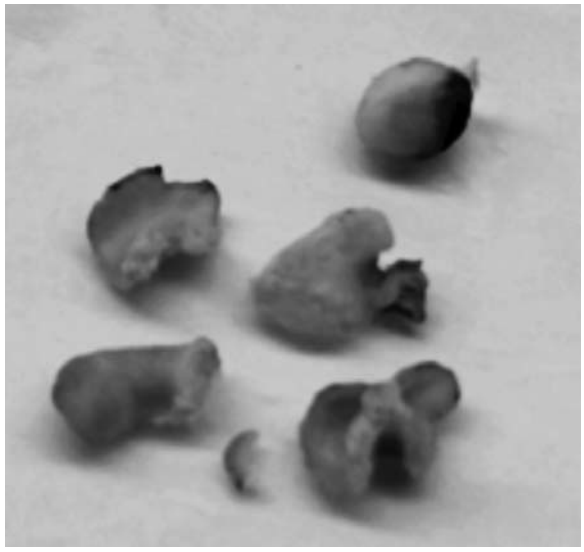

Fig.5 Callus from leaves explantsin 0.5 mg/l 2, 4-D and 0.1 mg/l NAA

In our study highest callus production was observed from hpocotyl explants of Radish variety Beeralu on MS basal medium supplemented with 0.5mg/l 2,4-D. Matsubara and Hegazi (1990) was observed callus initiation and growth and plantlet regeneration from 8 radish cultivars (6 Japanese radishes, one Chinese and one small radish (Comet)). Highest callus yield was obtained on basal medium containing 0.1 mg 2, 4-D/l and 1.0 mg benzyladenine (BA)/l for 2 Japanese radishes and 0.1 mg naphthalene

acetic acid/l and 1.0 mg kinetin/l for Comet. According to those results callus induction media was varied according to radish cultivars.

Regeneration ability of callus from selected explant on different media

When consider mean highest regeneration (6.6 shoots/explants) ability from hypocotyl calluses were exhibited in treatment 4 (2.5 mg/l BA + 0.1 mg/l NAA, Fig.6). affected from types of cultivar. Therefore results of this study can be change according to types of cultivars.

Table 1:
Mean comparison of regenerated shoots from callus in hypocotyl explant in different media

Treatments (BA + 0.1 NAA) mg/l	Number of shoots from explant (0.5 cm diameter callus)
1.0 (T_1)	0.6^{d}
1.5 (T_2)	3.2^{bc}
2.0 (T_3)	4.4^{b}
2.5 (T_4)	6.6^{a}
3.0 (T_5)	2.4^{c}

It is significantly different from other treatments. Treatment 3 (2.0 mg/l BA + 0.1 mg/l NAA, Fig.7) was best next to treatment 4 and it is same as treatment 2 (1.5 mg/l BA + 0.1 mg/l NAA, Fig.8). Treatment 5 (3.0 mg/l BA + 0.1 mg/l NAA) was same as treatment 2. So Treatment 2,3 and 5 were similar to each other. But treatment 1 (1.0 mg/l BA + 0.1 mg/l NAA) revealed lowest regeneration (0.6 shoots/explants) ability.

Sachiko and Hegazi (1990) reported plant regeneration from radish callus for the first time. There are contradictory reports on the effects of phyto hormones on the callus initiation of different plants (Mohammad *et al*, 2009) 2, 4-D is a growth regulator essentially needed for callus initiation and growth in most of the in vitro culture studies but it limits regeneration of plantlets (Mohammad *et al*, 2009). Furthermore, for regeneration purposes callus must be transferred to a 2, 4-D free medium with balanced combinations of cytokinin and one another auxin. Hypocotyl explants had a relative superiority for callogenesis in comparison with the other explants. There are several reports that diverse explants such as flower, leaf, petiole, stem, cotyledon, shoot-tip and cotyledon have beenemployed for callus initiation of different plant species (Mohammad *et al*, 2009). However hypocotyl explants showed the premium callogenesis in radish. Furthermore, hypocotyl explants were reported as the most suitable explant for in vitro culture purposes especially for callus initiation in broccoli, red cabbage and mustard. In some cases and for some genotypes of radish, concomitant production of callus and plantlets from hypocotyl explants were reported as well (Mohammad *et al*, 2009).

Mohammad *et al*, 2009 reported that variations in shoot and root production of two rapeseed (*Brassica napus*) cultivars were due to the differences in the internal levels of auxin and cytokinin. In fact differences in tissue culture responses and organogenesis would be dependent on the different potential of explants for internal hormones metabolism. Findings of this experiment are initial steps toward in vitro culture multiplication as well as a trend into enhancing a genetic engineering potential of this plant in this variety in Sri Lanka. However, in the future there is need for novel and accurate protocols for optimization of plant regeneration from these explants.

According to Kim *et al*, 2001 to direct regeneration cotyledon explants from four-day old seedlings were suitable for the effective induction of shoots on Murashige and Skoog's (MS) medium containing 3.0 mg/L kinetin and also determined that it was essential to include 1 - to 2-ram petiole segments with the cotyledons for efficient induction. But in present study hypocotyl explant was the best explant for regeneration from calluses on MS basal medium with 2.5mg/l.

Matsubara and Hegazi, 1990 was observed shoots were regenerated from callus on MS basal medium containing 0.1 or 1.0 mg BA/l. Although callus was obtained in all 8 cultivars (6 Japanese radishes, one Chinese and one small radish (Comet), shoots and plantlets were regenerated only from 3 cultivars; Moriguchi, Nerima Shirinaga and Comet. According to those results regeneration from calluses also affected from types of cultivar.

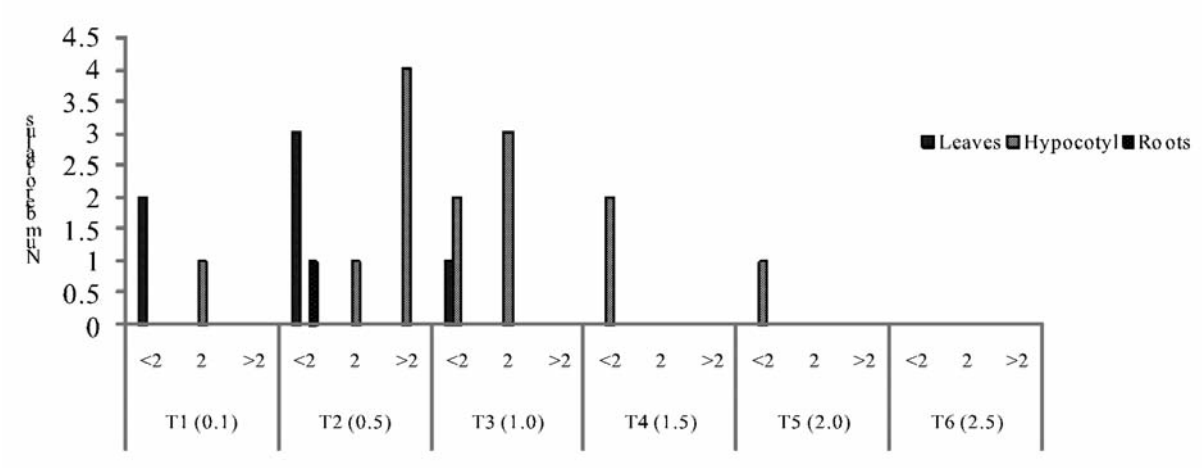

Fig. 2: Callogenesis from different explants in different hormone combination

Fig. 6: Shoot regenerated from hypocotyl explant in treatment 4 (2.5 mg/l BA + 0.1 mg/l NAA)

Fig. 7: Shoot regenerated from hypocotyl explant in treatment 3 (2.0 mg/l BA + 0.1 mg/l NAA)

Fig. 7: Shoot regenerated from hypocotyl explant in treatment 3 (2.0 mg/l BA + 0.1 mg/l NAA)

Fig. 9: Shoot regenerated from hypocotyl explant in treatment 1(1.0 mg/l BA + 0.1 mg/l NAA)

Conclusion

Hypocotyls showed to be better explants for callogenesis and regeneration. Maximum callogenesis was noted on MS medium supplemented with NAA (0.1 mg/l), 2, 4-D (0.5 mg/l).

Maximum regeneration was observed on MS medium containing 2.5 mg/l BA and 0.1 mg/l NAA. This study is a baseline to carry further research on radish variety Beralu for improvement by using gene transfer technology and make high yield variety

References

Curtis S, I, 2003, The noble radish: past, present and future, Trends in Plant Science, Vol.8 No.7, pp 305 - 307

Matsubara, S, Hegazi, H, H, 1990, Plant regeneration from hypocotyl callus of radish, Horticultural Science, Vol. 25 No. 10 pp. 1286-1288

Mohammad, A, Mohammad, B, H, 2009, The Effects of Different Culture Media on the Callus Production of Radish (Raphanus sativus L.),Romanian Biotechnological Letters,Romanian Society of Biological Sciences,Vol. 14, No. 4, 2009, pp. 4519-4523

Dahanayake, N, Xiao-Lu Chen, Fu-Cheng Zhao, Yue-Sheng Yang, Hong Wu ,2010,An Efficient In Vitro Propagation System for Purple Cone-Flower (Echinacea Purpurea L.)Journal of Tropical Agricultural Research & Extension 13(2)

Na Ryung Kim , Gynheung An , and Min Chul Park, 2001, High-Frequency Regeneration and Transformation of *Raphanus sativus*, Journal of Plant Biology, Vol 44(4), pp 231-235

Sachiko ,M and Hegazi H, H, 1990, Plant Regeneration from Hypocotyl Callus of Radish, Retrieved April 23, 2013 from

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Seed development with increasing carpel number in Sesame *(sesamum indicum)* and its possibility in plant breeding

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Abstract: Genetic diversification based on morphological attributes in sesame grown in Jaffna district was critically examined in this study. The results showed that, seed number is positively correlated with number of carpels in sesame capsule. Higher seed number was obtained in four carpel (82.06) and five carpel (84.94) types, Multilocules and multicapsules of the sesame are the major attributes to increase the yield. However, five carpel and six carpel capsule contain sesame cultivar are not recommended yet in India or Sri Lanka.

Keywords: sesame, multicapsule, multilocule, carpel type, locule number

Introduction

Sesame (*sesamum indicum*.L) is a member of order tubiflorae, pedaliaceae, perhaps the oldest oil seed known and used by human beings. It grows well in tropics and warm sub tropics. Sesame contains high oil (38-54%), protein (18-25%), calcium, phosphorous and oxalic acid content and excellent qualities of seed oil and meal. Thus, it is described as the "queen of oil seeds". Sesame contains lignans (sesamin, sesaminol and sesamolinol), which have antioxidant functions, so it resisting the oxidation. Owing to this reason, sesame oil has longer shelf life (Ram and Singh 2003).

Capsule arrangement in sesame is either monocapsular or multicapsular. The character is governed by a single gene, and the recessive allele produces triple capsules. Capsules form from flowers in the leaf axil from about 4–6 node pairs to the top of the plant (Ram and Singh. 2003).

Multilocules and multicapsules of the sesame are the major characters in increasing yield. Anyhow in Sri Lanka sesame variety UMA is the multicapsule white seeded cultivar. However sesame varieties with five carpel or six carpel capsules not recommended yet in Sri Lanka and India. Thus, our research project focused on studying the above characters to aid in utilizing these characters in sesame cultivar selection.

Ten crosses have been made with the parental combinations of 4×8, 8×8 and 4×8 locular parents. Progenies of these crosses were evaluated for assessing the genetic nature of the locule character in Sesamum. F1 from all types of segregation was 4L. The F2 segregation fitted to the 3:1 ratio of 4L versus multiloculed plants (Padmasundari et al., 2010).

Increasing of carpel number per capsules is the important consideration for increasing yield, so increasing carpel number as a theme of plant breeding will explore the high yielding varieties. This paper analysis sesame capsules of two carpel, three carpel, four carpel, five carpel and six carpel for seed development to aid future breeding programme.

Materials and Methods

Sesame plants with different carpeled nature were observed in farmer fields. From these plants, three types of plants having different combination of carpel plants are selected and grown again in plant to row basis for about 4 generation. The study site was located in the department of agricultural biology field, university of Jaffna and agriculture department field, thirunelvely in the northern dry zone of Sri Lanka during Maha season 2011.

Types of cultivars used in these experiments are

1. V1:-producing 4,5and 6 carpel capsules
2. V2:-producing bicarpillate capsules
3. V3:-producing 3 and 4 carpillate capsules

Four replicates for each sesame cultivar were used. In each replicate three plants were selected, in each plant 3 capsules were selected from the middle region. Length, width and seed number of these capsules were measured. Ten capsules from each capsule type (2carpels, 3carpels, 4carpels, 5carpels, and 6carpels) was collected from each replicate. Length, width and seed number were recorded.

Climatic parameters

The study site was located in Jaffna district, thirunelvely in the dry zone of Sri Lanka. The study site was located nearest to the meteorological station, thirunelvely, Jaffna, Sri Lanka. Meteorological parameters of the study site (minimum, maximum temperature, rainfall, relative humidity) were obtained from this meteorological station.

Table 1: Climatic parameters

Climatic parameters	Dec.	Jan.	Feb.	Mar.
Maximum temperature (c)	30	29.6	31	33.3
Minimum temperature (c)	22.8	20.3	22.1	24.1
Mean temperature (c)	25.8	24.9	26.5	28.7
Relative Humidity	81	75	66	71
Rain fall	295.1	6.4	0.2	8.3

Statistical analysis

Data were analyzed using analysis of variance (ANOVA) and mean comparison made with LSD, and the statistical significance level was set up at $p \leq 0.05$. Analysis were conducted with the SAS (SAS, 1993) and graphs were prepared with Microsoft excel software.

Results and Discussion

Table 3: Thousand seed weight of seeds in each carpel type (in grams)

Treatment	Mean
2c	3.2a
5c	3.0ab
4c	2.9b
6c	2.9b
3c	2.8b

Table 4:
Seed yield of different carpel type

Capsule type	Seed yield per capsules (gram)	No of seed bearing locules per capsules	No of seeds per locule	1000 seed weight
2c	0.2004	4.0	15.76	3.18
3c	0.2242	5.9	12.85	2.97
4c	0.2402	6.2	13.39	2.91
5c	0.2453	7.5	11.36	2.88
6c	0.2196	7.6	10.47	2.76

As shown in figure 1, seed number is positively correlated with carpel type. Two carpel capsules have low seed number (63.13), it is widely used in Sri Lanka. Five carpel capsules have the highest seed number (84.944) and the seed number of four carpel capsules (82.055) is not significantly different from that. Thus, according to the seed number, four and five carpel sesame capsules are ideal types for cultivation.

Thousand seed weight of two carpel (3.2 g) capsules is the highest and it was significantly differ from that of all the other types. However, thousand seed weight of five carpel capsules is also not so par from two carpel types (Table 3).

As depicted in the figure 2, bicarpillate capsule have percentage of seed fill of 100%. This is because bicarpillate capsules contain seeds in all locules. Percentage seed fill in tricarpillate capsule is 97.9% because tricarple contain six locules and most of this type of capsules contains seeds in all locules but some tricarpel capsules have seeds only in five locules. Compared to tricarpillate capsules, qudricarpillate capsules (77.1 %) have tremendously low percentage of seed filling. However, percentage seed filling of qudricarpillate capsules and five carpillate capsules (75.0%) are not significantly different.

Another tremendous reduction was observed from five carpel to six carpel this may be because of lack of pollen availability to fertilize all the ovules. Five carpel and four carpel capsule have more seed yield per capsule, five carpel capsule (0.2453g), four carpel capsule (0.2402g) (Table 4).

Three sesame capsules are found per leaf axil (Fig. 3 C and D). Most triple capsule lines have single capsules at the bottom and top of the plant and rarely have three capsules in every node. Axillary capsules on triple capsule lines are rarely the size of the central capsules and have fewer seeds with lower seed weight, resulting in lower seed weight per capsule. The axillary capsules also have less shatter resistance, and thus, they lose more seeds, while drying down. In the wind, leaves act like shock absorbers as the plants rub against each other, and when the leaves drop, the triple capsules rub off much easier than the single capsules. single capsule lines have more yields in the combine bin than triple capsule lines and Since all of the present Sesame varieties are single capsule in the US (Langham, 2008). So in mechanizing sesame cultivation monocapsule multilocule lines, may be give considerable yield.

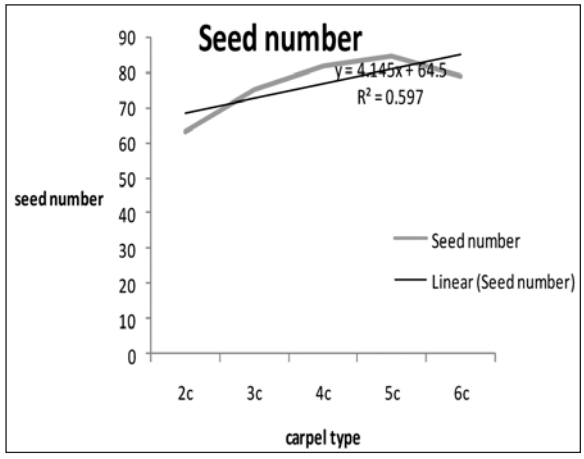

Figure 1:
Seed number related to different carpel type

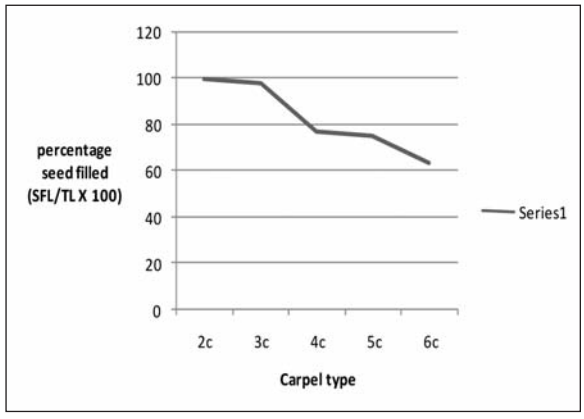

Figure 2:
Percentage seed fill related to different carpel type (seed filled locule number (SFL), total locule number(TL))

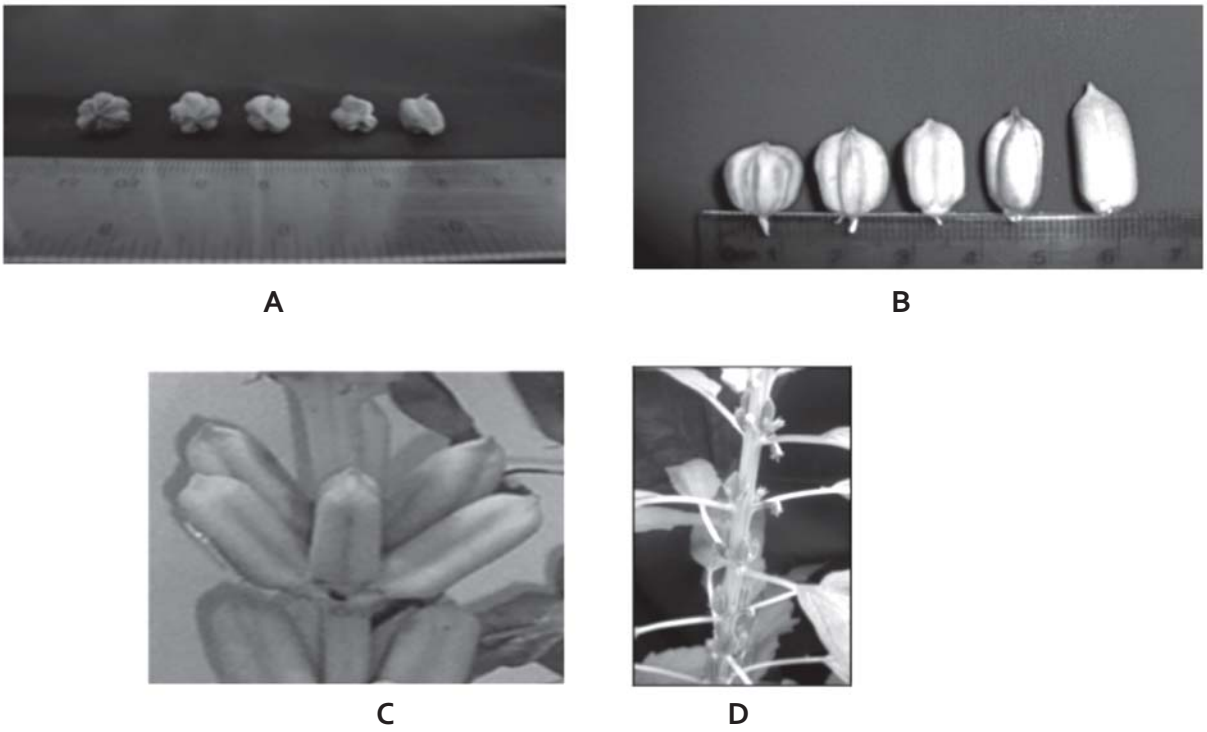

Figure 3: (A) Front view, (B) Lateral view of different carple tpes (C) multi-capsuleline character and (D) leaf arragement of the Multi-capsule line.

Conclusion

This experiment reveals that seed number is positively correlated with number of carpel in sesame capsule. Higher seed number was obtained in four (82.1) and five (84.9) carpel types. Two carpel capsules contain very low (63.1) seed number, significantly vary from all the other types. However, two carpel varieties are usually recommended in Sri Lanka

Thousand seed weight of two carpel (3.2 g) capsules is the highest and significantly differ from all the other types, Thousand seed weight of five (2.9 g) carpel capsule also not so par from two carpel types

With regard to seed number, four carpel (8 locules), five carpel (10 locules) sesame capsules are the ideal types although five carpel sesame is still not India or Sri Lanka. However, to produce high yielding lines more breeding experiments are needed.

References

Baydar, H. 2005. Breeding for the improvement of the ideal plant type of sesame, Vol: 124 (3), pp- 263–267

Ram and Singh, H.G. 2003. Crop breeding and genetics, Kalyani publishers, pp 420-436.

IPGRI, NBPGR 2004. Descriptors for Sesame (Sesamum spp), International Plant Genetic Resources Institute, Rome, Italy; and National Bureau of Plant Genetic Resources, New Delhi, India. pp. 28-46

Langham D.R. 2007. "Phenology of sesame," In: Janick, J. and

Whipkey, A. (Ed.), Issues in New Crops and New Uses, ASHS Press, Alexandria,VA, pp. 144-182.

Pamasundari, M., Kamala, T. and Rao, y.v. 2010 Genetics of locules in sesamum indicum L. Asian journal of Agricultural Sciences, 2(4):164-167.

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Carbon Credit Concept in Rice Production in Sri Lanka

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Abstract: Increasing emission of carbon dioxide, the most important green house gas to the environment is a serious threat in considering global warming. In today's scenario global warming is costing lot of money and energy which lead for starting a global business of exchanging carbon between businesses called carbon credit. This carbon credit system works between different industries such as manufacturing, construction etc but not with agriculture. In this paper contribution of rice cultivation to the carbon credit is discussed. This paper also suggests an alternative income to the rice farmers through carbon credit in order keep viable the rice cultivation in Sri Lanka.

Keywords: Carbon credit, Carbon dioxide, Photosynthesis

Introduction

A carbon credit is a generic term for any tradable certificate or permit representing the right to emit one tone of carbon dioxide or the mass of another greenhouse gas with a carbon dioxide equivalent to one tonne (Investopedia, 2013). One carbon credit allows one tonne of carbon dioxide or a corresponding amount of other greenhouse gases to be discharged in the air. Businesses that are over their quotas must buy carbon credits for excess emissions, while those below can sell their remaining credits. This exchange of credits between businesses has encouraged carbon trading globally. These credits can be exchanged between businesses or bought and sold in international markets at prevailing market price at two exchanges, namely the Chicago climate exchange and the European climate exchange. The multi-commodity exchange of India may soon become the third exchange in the world to trade in carbon credits (Gupta, 2011). Even though Rs. 6000.00 is expected by the environmentalists for one ton of carbon credit, currently it is Rs 2000.00 (Carbon trade exchange, 2012).

The Kyoto Protocol has created a mechanism under which countries that have been emitting more carbon and other gases (greenhouse gases include ozone, carbon dioxide, methane, nitrous oxide and even water vapor) have voluntarily decided that they will bring down the level of carbon they are emitting to the levels of early 1990s. This showed a way that green house gas emitting organizations can tie up with developing nations and help them to exchange the carbon credits. Every year European companies are required to meet certain norms, in the coming years there will be a lot of carbon credit deals (Bhatia et al, 2006). However this carbon credit concept is currently developed mainly for the manufacturing industries. This is the time the agriculture sector to think of its contribution to the environment in terms of carbon credit. It can be easily illustrated by comparing two alternative options such as rice cultivation and clay manufacturing at a certain location. Clay manufacturing emit carbon dioxide (0.000186 tonnes of carbon dioxide /m^2/per year) to the environment where as rice cultivation consume carbon dioxide from environment (Brick Development Association. 2013).

Rice production in Sri Lanka can generate significant amount of tradable can carbon credit and this should be enlightened globally in order to transfer the benefit of this to farmers. Rice is the single most important crop occupying 34 percent of the total cultivated area in Sri Lanka (Department of Agriculture, 2012). Rice is cultivated in two seasons namely Maha and Yala. On average 560,000 ha are cultivated during Maha and 310,000 ha during Yala

making the average annual extent sown with rice to 870,000 ha. Due to high cost of production (cop) and stagnant productivity, viability of paddy cultivation in Sri Lanka is in question. Nearly 1.8 million farm families are engaged in paddy cultivation in Sri Lanka to produce 2.7 million tons of rough rice annually and supply 95 percent of the domestic requirement. In commercial level rice cultivated with higher cop of Rs 30.50 per Kg. This cost will be even higher if the there is no fertilizer subsidy scheme. The cost of labor, farm power and tradable inputs constitutes 55%, 23% and 23% respectively. On the other hand the domestic price of rice on par with Thai A1 super (the cheapest in the world market) is higher by Rs 7500.00. per tonne (Department of Agriculture, 2012). Razmy and Ahmed (2005) showed there is very less return to investment for farmers in rice cultivation due very high cop in Sri Lanka and it will decrease further with time. To keep viable the rice production in Sri Lanka, increasing the net profit of the farmers is an urgent need. Sri Lankan government is trying to achieve it through increasing the productivity but not succeeded yet. Decreasing the cop is another move but due to the increasing trend of the cost of inputs, it is not possible. Alternatively in this paper possibility of selling the carbon credit earned through rice cultivation is examined to increase profit of the farmers by estimating the carbon credit generated by the rice production in Sri Lanka.

Estimation of carbon credit

The net amount of carbon dioxide consumed by rice plants is estimated using different research studies on rice. The total leaf area index varies with the stages of the rice plant and remains constant after 9 weeks (Vaesen *et al,* 2001). In general the average number of leaves per rice plant is eight (Misra, 1955). The average leaf area per rice plant over its lifespan and net carbon dioxide consumption for photosynthesis for rice were reported 33.33 cm^2 and 7.395 μ mol m^{-2} s^{-1} respectively (Safeena *et al*, 2006). The total leaf area per plant is calculated as (leaf area X average leaf /plant) 266.64 cm^2. The average number of rice plants per acre was reported 792,000 (Gomez and Palaniswamy, 1974). The total leaf area per hectare of rice is estimated 52,161.18 m^2 (266.64 X 2.47 X 792000). Therefore the Carbon dioxide consumption rate by one hectare of rice cultivation is 385,731.95 μ mol s^{-1} (7.395 μ mol m^{-2} s^{-1} X 52,161.18336 m^2). In average, rice photosynthesizes for 11 hours and lifespan is 115 days (Misra, 1955). The Carbon dioxide consumption of one hectare rice cultivation for a day is estimated as 15275 mol (385,731.95 μ mol s^{-1} X 11 X 60 X 60 S) and the Carbon dioxide consumption in 115 days by one hectare rice cultivation is 77.3 metric tons (15275 mol X 115 X 44 / (1000 X 1000)). Therefore in one year the net carbon dioxide consumed by rice cultivation in Sri Lanka is 67,251,000 metric tons.

Results and Discussion

If the carbon credit concept is applied to the rice production in Sri Lanka, the claimable amount for 67,251,000 metric tons of carbon credit is Rs. 134.5 billion per year. This income from carbon credit could be shared among the farmers by subsidizing the farming inputs against this income. This move will protect the rice cultivation in Sri Lanka by increasing the rate of return to the investment. Alternatively if these all rice cultivation land is used for clay brick industry, there will be 1,618,200 metric tons emission of carbon dioxide per year. This illustrates the benefit to the environment by the rice cultivation over the brick industry. Therefore the Sri Lankan poor farmers should be given their share on carbon credit for their job of not emitting carbon dioxide but consuming through cultivation. This concept of carbon credit in agriculture need to be taken consideration by the international policy makers and this benefit should be enjoyed by the farmers.

References

Bhatia, Jatinder, S. & Harsh Bhargava. (2006) An insight into carbon trading: Understanding the behaviour of emissions market with a financial perspective. *The ICFAI Journal of Applied Finance*. 12(11). P. 59-69

Brick Development Association. (2013) Sustainability. [Online] Available from:
[Accessed: 10th April 2012].

Carbon trade exchange. (2012) Carbon trade exchange enables companies to easily and cost effectively buy and sell voluntary carbon credits known as verified emission reductions. [Online] Available from: [Accessed: 14th January 2012].

Department of Agriculture. (2012) Rice production. [Online] Available from: . [Accessed: 15th January 2012].

Gomez, K. A. & Palaniswamy, K. M. (1974) Variability in leaf area of rice. *Experimental Agriculture* 10(3) P.169-175.

Gupta, Y. (2011) Carbon credit: A step towards green environment. *Global Journal of Management and Business Research*. 11 (5). P.17-19.

Investopedia. (2013) Definition of carbon credit. [Online] Available from: .
[Accessed: 12th January 2012].

Misra, G. (1955) Effect of short day length of three medium-early varieties of rice of Uttar pradesh. *Photoperiodism in Rice.* 21(1) p. 1-9.

Razmy, A.M. & Ahmed, A.N. (2005) Profitability of paddy production in the coastal region of the Ampara District. *AGRIEAST.* 4(1) p. 64-74.

Safeena, M.I.S, Bandara, D.C. & Sumanasinghe, V.A. (2002). Effect of salt stress on leaf area, rate of photosynthesis and proline accumulation of rice varieties at different growth stages. *Tropical Agricultural Research* 14 p. 87-96.

Vaesen, K., Gilliams, S., Nackaerts, K. & Coppin, P. (2001) Ground-measured spectral signatures as indicators of ground cover and leaf area index: the case of paddy rice. *Field Crops Research.* 69 (1) p. 13-25.

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Comparative analysis of the egg quality traits in different chicken genotypes in the Dry Zone of Sri Lanka

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Abstract: A study was done to investigate the egg quality traits of different chicken genotypes in the dry zone of Sri Lanka. Results revealed that highly significant differences exist for these quality traits between the genetic groups of chicken ($P<0.05$). The exotic chicken showed the significantly highest values for all the egg quality traits except fertility. However, the naked-neck genotype shows good qualities on the shell thickens and fertility and showed medium values for all other traits. The present findings therefore suggest that the naked-neck is superior than other genotypes next to exotic chicken.

Keywords: Exotic chicken, Genotypes, Fertility, Naked-neck chicken

Introduction

The analyses of the egg structural traits are essential for an understanding of egg quality, fertility, embryo development, hatchability and diseases of the poultry (Banerjee, 1992). Economically important egg quality traits such as weight, size, yolk and albumen contents are quantitative traits with continuous variability (Das 1994). Egg weight is one of the important phenotypic traits which influence egg quality and reproductive fitness of the chicken parents (Islam *et al*., 2001; Farooq *et al*., 2001). It is obvious that beneficial egg quality traits are of immense importance to poultry breeding industries (Bain, 2005). Further, embryonic development of hen's egg is dependent on traits like egg weight, yolk and albumen weights, genetic line and age of the hen (Finkler *et al*., 1998; Onagbesan *et al*., 2007). Subsequently, effects of feed (Adedeji *et al*., 2008; Shapira, 2010), hormone (Guzel *et al*., 2009) and housing system (Pohle and Cheng, 2009; Sossidou and Elson, 2009; Wang *et al*., 2009) on egg composition and its quality have been investigated in various countries. However, in Sri Lanka no studies have been undertaken yet to evaluate the egg quality traits of different chicken genotypes. In this context, the present study was planned to analyze the effect of different genotypic group of chicken on egg quality. The results of the study can be used to improve the genetic potential of existing local genetic group of chicken as these local genotypes are important component in the mixed farming system in Sri Lanka.

Materials and Methods

Egg collection

A study was done to evaluate the egg quality traits of different chicken genotypes. Village chicken, naked-neck, Hy-line white (exotic chicken) and cross-bred chicken (Exotic x local chicken) were selected for the analysis. The samples were collected from various locations in Batticaloa and Ampara districts. A total of 50 fresh eggs were collected from each genotypic group. Eggs were collected from the chicken flock 7-10 months after on-set of laying. The village chicken and naked-neck chicken were reared under semi-intensive system while the exotic and cross-bred chickens were reared under intensive system (Deep litter system). The cockeral: hen ratio of the different genotypic group was 1:10.

External and internal egg quality traits

For this study egg weight, egg length, egg width, egg volume, shell weight, shell thickness, yolk weight, albumin weight and fertility were measured. From these basic parameters egg shape index and yolk:albumin ratio were calculated. The eggs were numbered first and then weighed on an electronic balance to determine their weights. Subsequently, length and width of the eggs were measured manually and the egg volume was determined using the water displacement method. Each egg was broken on a table and its contents were poured into a plate. Then the yolk was separated from the albumen with the help of a spoon and weighed while the albumin weight was calculated by subtracting yolk weight and shell weight from the egg weight. Egg shape index, fertility percent and yolk:albumin ratio were calculated using the following formula (Olawumi and Ogunlade, 2008):

a) Egg shape index (%) = Egg width/Egg length × 100

b) Fertility (%) = Number of fertile eggs/Total number of eggs × 100

c) Yolk: Albumin ratio = Yolk/Albumin

Statistical analysis

Mean, standard deviation (SD), analysis of variance (ANOVA) and least significant Differences (LSD) were computed using the SAS (version 9.0). Data on various external and internal egg quality traits were subjected to these statistical procedures to detect the significance of difference between the genetic groups of chicken under study.

Results and Discussions

The mean values of exterior egg quality of traits and interior egg composition traits of different chicken genotypes are given in Tab 1 and Tab 2.

Exterior quality of eggs

Exterior egg quality is defined as texture, colour, soundness, cleanliness and shape of the shell. The shell of each egg should be smooth, clean and free of cracks and eggs should be uniform in colour, size and shape (Coutts *et al.*,2006).

Table 1:
Exterior egg quality traits of different chicken genotypes

Exterior egg quality traits	Village chicken	Naked-neck chicken	Exotic chicken	Exotic x Local chicken
Egg shape index (%)	67.21±0.56^{a}	72.67±0.58^{b}	79.21±0.64^{c}	73.45±0.67^{b}
Egg weight (g)	43.43±1.91^{a}	48.61±1.28^{b}	54.83±2.64^{c}	50.44±1.29^{d}
Specific gravity	1.07±0.01^{a}	1.12±0.02^{b}	1.17±0.01^{c}	1.11±0.01^{b}
Fertility (%)	85.35±2.12^{a}	91.8±1.64^{b}	86.46±3.22^{a}	91.4±1.33^{b}

Numbers followed by different letters are significantly different (P<0.05)

Egg shape index

The results of the study showed that the genotypic effect on shape index was significant (P<0.05). Egg shape index was significantly higher (p<0.05) in exotic chicken (79.21±0.64%). As egg weight is high the shape index also higher in exotics. Egg shape index is directly proportional to egg weight (Pandey *et al.*, 1986). Lower shape index was recorded in village chicken (67.21±0.56%). Differences between strains for egg shape index have been reported by many authors (Dottavio *et al.*, 2001; Chatterjee *et al.*, 2007). Higher egg shape index (78%) was recorded in indigenous chicken in Bankaladesh (Saiful and Ripon, 2010). Standard errors for egg shape index were quite small illustrating the homogeneity of egg shape in all the chicken genotypes. It has to be mentioned that opposite results were obtained by Fikry Amer (1972) and Merat *et al.*, (1983), who reported a higher shape index for eggs of the local breeds in Bankaladesh than for the commercial Rhode Island Red strain.

Egg weight

The breed effect on egg weight was highly significant ($p<0.05$). The egg weight was significantly higher ($P<0.05$) in exotic chicken (54.83±2.64g) than other chicken genotypes. Comparatively lowest egg weight was recorded for village chicken (43.43±1.91g). The lower body weight of the village chicken is the major reason for smaller eggs produced. The similar observation was made by Islam (2006), Chatterjee *et al.,*(2006; 2007), Niranjan *et al.*, (2008), Olawumi and Ogunlade (2008) Jones *et al.*, (2010) and Tixier-Boichard *et al.*, (2006) in various studies at different regions. The very low egg weight in local breeds in comparison to commercial strains is a well-known fact and is reported in literature by different authors (Parmar *et al.*, 2006; Harms and Hussein, 1993; Fikry Amer, 1972; Tixier-Boichard *et al.*, 2006). This difference is not surprising as commercial strains have been submitted to important breeding pressure for egg weight improvement (Hocking *et al.*, 2003).

Specific gravity

Specific gravity was significantly higher ($p<0.05$) in exotic chicken (1.17±0.01) as the eggs of exotic chicken have higher weight and shape index. Therefore, the gravity was higher as the weight and volume are the determinants of specific gravity. Comparatively lowest value was recorded in village chicken (1.07±0.01).

Egg fertility

The effect of different genotypes on egg fertility was significant ($P<0.05$). Fertility percent was significantly higher ($P<0.05$) in naked-neck (91.8±1.64%) and cross-bred chicken (91.4±1.33%) as these both genotypes had medium egg size and weight and fairly high resistant to pathogens.

Albumin weight

In accordance with the results obtained on albumen weights differed significantly between breeds ($P<0.05$), with highest values being presented by exotic chicken eggs (31.72±1.33g). The present results confirm those obtained by Fikry Amer (1972), Tixier-Boichard *et al.*, (2006) and Offiong *et al.,* (2006). The reason for higher albumin weight is due to high value for egg weight. The correlation between total egg weight and albumen weight was strong and positive (Hartmann *et al.*, 2000; Suk and Park, 2001). Lowest albumin weight was recorded for village chicken (22.90±0.57).

Table 2: Egg composition traits of different chicken genotypes

Egg composition traits	Village chicken	Naked-neck chicken	Exotic chicken	Exotic x Local Chicken
Albumin weight (g)	22.90±0.57^{a}	25.33±1.32^{a}	31.72±1.33^{b}	27.94±1.87^{d}
Yolk weight (g)	15.17±1.58^{a}	15.52±1.66^{a}	17.01±1.54^{b}	17.61±1.22^{b}
Yolk: Albumin ratio	0.67±0.01^{a}	0.61±0.01^{b}	0.53±0.00^{c}	0.63±0.01^{b}
Egg shell weight (g)	6.07±1.38^{a}	8.61±1.12^{b}	6.76±2.05^{a}	6.44±1.20^{a}
Average egg shell thickness (mm)	0.52± 0.002^{a}	0.53± 0.003^{a}	0.49± 0.003^{b}	0.50± 0.006^{b}

Numbers followed by different letters are significantly different ($P<0.05$)

Yolk weight

Breed effect on yolk weight also proved to be differed significantly ($P<0.05$) (Table 3.2). However, no statistical difference was observed between village (15.17±1.58g) and naked-neck chicken (15.52±1.66g) and exotic (17.01±1.54g) and cross bred chicken (17.61±1.22g). In literature, similar observation was reported by Fikry Amer (1972), Merat *et al.* (1983) and Parmar *et al.* (2006). The correlation between yolk weight and total egg weight was strong and positive

(Hartmann *et al*., 2000; Suk and Park, 2001; Offiong *et al*., 2006).

Yolk: Albumin ratio(Y: A ratio)

The calculated Y:A ratio was significantly highest in village chicken (0.67±0.01[a]) which is close to the ratio reported for cross-bred chicken (0.63±0.01). However, lowest values for yolk:albumin ratio was recorded by Suk and Park (2001) who recorded a Y:A ratio of 0.55 in Korean Native Chicken and 0.38 commercial egg-type chicken. As for yolk percentage, the Y:A ratio showed a strongly negative correlation with total egg weight (Suk and Park, 2001). Here it is important to line out the great importance of yolk proportion in egg processing industry as it is linked to higher dry matter content and to a higher content of essential fatty acids (Benabdeljelil and Merat, 1995).

Egg shell weight

Egg shell weight was significantly highest (P<0.05) in naked-neck (8.61±1.12g). No significant difference (P<0.05) was observed on egg shell weight among genotypes except naked-neck. A strongly positive correlation was observed between egg shell thickness and total egg weights in various studies (Suk and Park, 2001). However, the results of the present study were not agreed the correlation report. Comparatively lowest egg shell weight was recorded in different chicken genotypes in other countries (Fikry Amer, 1972 and Harms and Hussein, 1993). Interestingly, according to these authors, no relation existed between egg shell weight and total egg weight. Correlation co-efficient between this trait and total egg weight was in the present study strongly negative, which is in agreement with Das *et al*., (2006).

Average egg shell thickness

Average egg shell thickness was also influenced by breed. Egg shell thickness was significantly higher (P<0.05) in naked-neck (0.53± 0.003) and village chicken (0.52± 0.002), No significant difference (P>0.05) was observed between exotic and cross-bred chicken for egg shell thickness. In general, calcium is the determinant factor of shell thickness. In commercial exotic chicken calcium will be supplemented with feed. However, the local chickens selected for this study were collected from semi-intensive farms. In semi-intensive system the chicken has access to get diverse mineral during their scavenging. This might lead to more calcium and more shell thickness in eggs. In literature it is reported that in commercial strains shell thickness is higher than in local breeds (Suk and Park, 2001).

Conclusion

The exotic chicken showed higher values for egg exterior quality traits and egg composition traits. However, naked-neck chicken showed higher values for hatchability, egg shell thickness and egg shell weight. Naked-neck chicken also noted for medium quality in terms of exterior and composition traits. Therefore, there is a potential to improve naked-neck chicken population through proper selection and breeding.

References

Adedeji, O.S., Farinu, G.O., Olayeni, T.B., Ameen, S.A. and Babatunde, G.M, (2008). Performance of egg quality parameters of laying hens fed different dietary inclusion levels of bitter Kola (*Garcinia kola*). *Res. J.Poul. Sci.* **2(4):** 75-77.

Bain, M.M. (2005). Recent advances in the assessment of egg shell quality and their future application. *World's Poult. Sci. J.* **61**: 268-277.

Banerjee, G.C. (1992). *Poultry*. Mohan Primlani for Oxford and IBH Publ. Co. Pvt. Ltd. New Delhi, India. 191 pp.

Benabdeljelil, K. and Merat, P. (1995). Comparaison de types génétiques de poules pour une production d'oeufs locale: F1 (Fayoumi × leghorn) et croisement terminal ISA au Maroc. Ann. Zootech. **44**, 313-318.

Chatterjee, R.N., R.B. Rai, A. Kundu, S. Senani and S. Jai, (2007): Egg quality traits of indigenous breeds of chicken of Andaman. Indian Vet. J. **84**, 206-208.

Coutts, J.A., G.C. Wilson, S. Fernandez, E. Rasales, G. Weber and J.M. Hernandez, (2006): Optimum Egg Quality - A Practical Approach. 5M Publishing: Shefield UK, p 63.

Das, S.K. (1994). *Poultry Production*. CBS Pub. and Distributor, New Delhi, India. 232 pp.

Dottavio, A.M., Z.E. Canet, M. Alvarez, B. Creixell, R.J.Di Masso and M.T. Font, (2001): Productive traits in hybrid hens with Fayoumi maternal genotype. Arch. Latinoam. Prod. Anim. **9**, 57-62.

Farooq, M., Mian, M.A., Ali, M., Durranim, F.R., Asquar, A and Muqarrab, A.K. (2001). Egg traits of Fayoumi bird under subtropical conditions. *Sarad. J. Agri.* **17:** 141-145.

Fikry Amer, M., (1972): Egg Quality of Rhode Island Red, Fayoumi and Dandarawi. Poult. Sci. **51**, 232-238.

Finkler, M.S., Orman, J.B.V and Southerland, P.R. (1998). Experimental manipulation of egg quality in chickens:Influence of albumen and yolk on the size and body composition of near-term embryos in a precocial bird. *J. Comp. Physiol.* **168:** 17–24.

Guzel. S., Gunes, N., Yldz, H and Yilmaz, B. (2009). Effects of amylin on bone development and egg production in hens. *Poult. Sci.* **88:** 1719-1724.

Harms, R.H and S.M. Hussein, (1993): Variations in yolk: albumen ratio in hen eggs from commercial flocks. *J. Appl.Poult. Res*. **2**, 166-170.

Hartmann, C and M. Wilhemson, (2001): The hen's egg yolk: a source of biologically active substances. *World's Poult. Sci. J*. **57**, 13-28.

Hocking, P.M., M. Bain, C.E. Channing, R. Fleming and S.Wilson,(2003): Genetic variation for egg production, egg quality and bone strength in selected and traditional breeds of laying fowl. Br. *Poult. Sci*. **44**, 365-373.

Islam, M.A., Bulbul, S.M., Seeland, G and Islam, A.B, (2001). Egg quality of different chicken genotypes in summer and winter. *Pakistan. J. Biol. Sci*. **4:** 1411-1414.

Merat, P., A. Bordas., R. L'hospitalier., J. Portais and M. Bougon, (1983): Etude des particularités de la poule Fayoumi. III. Ponte, caractéristiques des oeufs, efficacité alimentaire et parametres physiologiques de poules Fayoumi, Rhode Island Red et F1 en batteries. Genet. Sel. Evol. **15**, 147-166.

Niranjan, M., Sharma, R.P., Rajkumar, U., Chatterjee, R.N., Reddy, B.L.N and Battacharya, T.K. (2008). Egg quality traits in chicken varieties developed for backyard poultry farming in India. *Livestock Res. Rural Dev*. **20:** 131-137.

Offiong, S.A., O.O. Ojebiyi., E.O. Moeses., B.I. Umoh and E.E.A. Offiong, (2006): Comparison of the morphometric characteristics of exotic commercial and local chicken eggs in the tropical environment. J. Anim. Vet. Adv. **5**, 1046-1049.

Olawumi, S.O and Ogunlade, J.T. (2008). Phenotypic Correlations Between Some External and Internal Egg Quality Traits in the Exotic Isa Brown Layer Breeders. *Asian J.Poult. Sci.* **2(1):** 30-35.

Onagbesan, O., Bruggeman, V., Desmit, L., Debonne, M., Witters, A., Tona, K., Everaert, N and Decuypere, E. (2007). Gas exchange during storage and incubation of Avian eggs: effects on embryogenesis, hatchability, chick quality and post-hatch growth. *World's Poult. Sci. J.* **63**: 557-573.

Pandey, N.K., Mahapatra, C.M., Verma, S.S and Johari, D.C. (1986). Effect of strain on physical egg quality characteristics in White Leghorn chickens. *J. Poult. Sci*. **21:** 304-307.

Parmar, S.N.S., M.S. Thakur., S.S. Tomar and P.V.A. Pilla, (2006): Evaluation of egg quality traits in

indigenous Kadaknath breed of poultry. Livestock Research for Rural Development, 18: article 32.

Pohle, K and Cheng, H.W. (2009). Comparative effects of furnished and battery cages on egg production and physiological parameters in White Leghorn hens. *Poult. Sci.* **88:** 2042-2051.

Saiful Islam and Ripon, K.D,(2010). Egg Quality Traits Of Indigenous, Exotic And Crossbred Chickens (*Gallus Domesticus* L.) In Rajshahi, Bangladesh. *J. Life Earth Sci*., Vol. 5: 63-67, 2010.

Shapira, N. (2010). Every egg may have a targeted purpose: toward a differential approach to egg according to composition and functional effect. *World's Poult. Sci. J.* **66**: 271-284.

Sossidou, E.N and Elson, H.A. (2009). Hens' welfare to egg quality: a European perspective. *World's Poult. Sci. J.***65**: 709-718.

Suk, Y.O and C. Park, (2001): Effect of Breed and Age of Hens on the Yolk to Albumen Ratio in Two Different Genetic Stocks. Poult. Sci. **80**, 855-858.

Tixier-Boichard, M., C. Joffrin., D. Gourichon and A. Bordas, (2006): Improvement of Yolk Percentage by Crossbreeding Between a Commercial Brown-Egg Layer and a local Breed, the Fayoumi. In: 8th World Congress on Genetics Applied to Livestock Production. Belo Horizonte, MG.Brasil.

Wang, X.L., Zheng, J.X., Ning, Z.H., Qu, L.J., Xu, G.Y and

Yang N. (2009). Laying performance and egg quality of blue shelled layers as affected by different housing systems. Poult. Sci. 88:1485-1492.

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Morphological Evaluation and Molecular Screening for Drought Resistance for Rice *(Oryza sativa L.)*

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Abstract: We screened 39 rice lines; 22 IRRI lines, 6 lines from International Rice Drought Nursery IRDN – 2007 (DSN group), 2 lines from Chinese drought tolerant Nursery (CNI group), 9 rice lines from Batalegoda and 5 local standard varieties for drought resistance under RCBD with three replicates. Morphological data revealed that, there was no significant difference (α=5%) between local varieties for the drought resistance but, IRRI lines showed significantly high leaf area index & root dry weight. Among the primers RM 208, RM 21, and RM 247, only RM 21 and RM 208 were showed polymorphism for drought. Bg 250 & AR 09/16 showed drought resistance with RM 208 and DSN22, DSN56, AR10/05, 11/723, DSN 40, DSN 54, DSN 37, AR 10/33, BG 94-1& DSN 2 showed drought resistance with RM 21 indicating the potential use of these primers to screen drought resistance.

Keywords: molecular screening, drought resistance, microsatellite markers, polymorphism

Introduction

Large areas of rice are grown under lowland and upland rain fed conditions. These areas respectively occupy 31% and 11% of the global rice-growing areas (IRRI, 2001).Several abiotic and biotic factors limit the rice production, among the abiotic stresses, drought is a serious limiting factor that reduces rice production and yield stability in rain fed ecosystems. Drought is estimated to frequently affect 19 -23 million ha of rice lands globally. Estimated global rice yield lost due to drought to be 18 million tons annually (4%) of total rice production (Kamoshita *et al* 2008). Drought stresses showed an increase in respiration and less photosynthesis reactions as it reduces the plant size, leaf area and turgidity of the plant. According to the rice ecosystem, rain fed lowland or upland; there are 3 types of drought terms of timing may be recognized, as vegetative stage drought, intermittent drought, and terminal drought. The major breeding objective in these ecosystems is to improve drought resistance in rice plants but, little progress has been achieved in improving yield under stress due to poor knowledge of the genetic control of drought resistance (Hanamaratti, 2007). Several putative traits contributing to drought resistance as root thickness, depth of rooting, root length, leaf rolling, tissue death, grain yield etc. were recorded (Ahmdi, 2007). Phenotypic selection for such traits is labour-intensive (Fukai, 1995). Marker Assisted Selection (MAS) serves as a tool for selecting such complex traits and allows breeders to track genetic loci controlling drought resistance traits, without having to measure the phenotype, thus reducing the need for extensive field testing over space and time.

Materials and Methods

Plant Materials

The tested lines and varieties were 22 rice breeding lines from IRRI, Philippines , 5 standard local varieties, 2 Chinese drought nursery rice varieties, 6 international drought rice nursery (IRDIN) varieties, and 1GSR inbred line from RRDI, Batalegoda (Table 01).

Morphological Evaluation

Morphological screening was conducted at the research field, Rice Research and development Institute, Bathalegoda. Selected accessions were grown under rain fed condition by giving natural water stress. The experiment was layout under RCBD with three replicates. Data were collected during the period of experiment.

Data Analysis

Morphological data was analyzed by using ANOVA procedure and means were separated by applying Duncan Multiple range Test (DMRT).

Molecular Screening

Genomic DNA was extracted from each rice varieties and lines following the CTAB "Miniprep" protocol (Saghai-Maroof et al. 1984). and DNA

Table 1:
Tested rice lines and varieties used for the study and their origin

Type of variety / line	Name of variety or line	Origin
IRRI Lines	IR83140-B-11-B, IR 83140-B-28-B, IR 83140-B-32-B, IR 83140-B-36-B,IR 83141-B-17-B, IR 83141-B-18-B, IR 83142-B-19-B, IR 83142-B-20-B,IR 83142-B-21-B, IR 83142-B-49-B, IR 83142-B-57-B, IR 83142-B-60-B,IR 83142-B-61-B, IR 83142-B-79-B, IR 83142-B-7-B-B, IR 83142-B-8-B-B, HHZ 12-Y4-Y3-Y1, HHZ 15-SUB-Y3-Y1,HHZ 5 SAL 10-DT1-DTI, HHZ 5 SAL 10-DT2-DTI, HHZ 5-T3-SAL3-DT1, HHZ 9-DT 7- SAL 2-DT1, HHZ 11-Y11-Y3-DT1, HHZ 17-DT 6-SAL3-DT1.	Philippine
Local standards	Bg 250, Bg 300, Bg 304, Bg 366, Bg 94-1	Batalegoda, Sri Lanka
IRDIN varieties	DSN 2, DSN 11, DSN 37, DSN 40, DSN 54, DSN 56	Philippine
Chinese drought tolerant var.	CNI 24, CNI 28	China
GSR inbred line	GSR-I- 0057	Batalegoda, Sri Lanka

Data Collection

Leaf area index (Measured in cm^2 using LiCor LI-3000 portable area meter), lengths (cm) of root and root ball, number of tillers and bio mass dry weight (g) of leaves, roots, shoots were measured at pre bloom stage. Also, days to 50% flowering and plant height at flowering stage (cm), were recorded. Leaf rolling was measured by using standard evaluation system for drought stress (IRRI, 1996) when plants show drought symptoms.

confirmation was done by using 0.8% agarose gel electrophoresis and PCR amplification for the genomic DNA was done by using three SSR primers (RM 208, RM 21, and RM 247). PCR was performed in a total volume of 15 µl containing 5 x PCR buffer mixtures 3.0 µl, 25 mM $Mgcl_2$ 2.4 µl, 10µm dNTPs 0.3 µl, 1.6 µl from 5 µm forward and reverse primers, 0.25 µl from 5U/µl Taq polymerase, 2.85 µl form sterile water and 3 µl of rice genomic DNA. PCR amplification occurred as initial denaturation at 94°C for 5 min, followed by

35 cycles at 94°C for 1 min of denaturation, primer annealing at 59.1°C for 1 min, primer extension at 72°C for 2 min and a final extension of 72°C for 5 min for RM 21, RM 208. For RM 247 primer, annealing at 55°C for 1 minwere used with same all the other steps. Amplified products were resolved in 3% agarose gels with ethidium bromide staining.

Results and Discussion

Morphological Evaluation

Among all the morphological characters, leaf area index and root dry weight showed the significant difference (α=5%), and among the all varieties IR 83142-B-21-B, IR 83140-B-36-B, HHZ 5-T3-SAL3-DT1 showed drought resistance over the others.

Figure 1:
Leaf area index–IRRI lines-The test is significant at α 5% level.

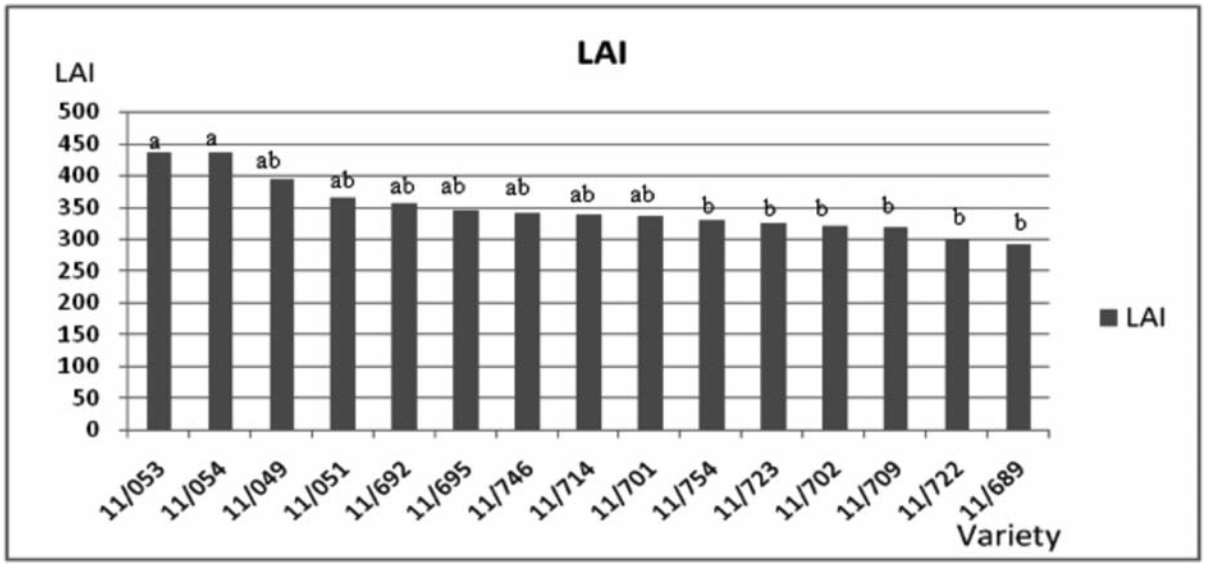


Figure 2:
Root dry weight – IRRI lines- The test is significant at α 5% level of significance

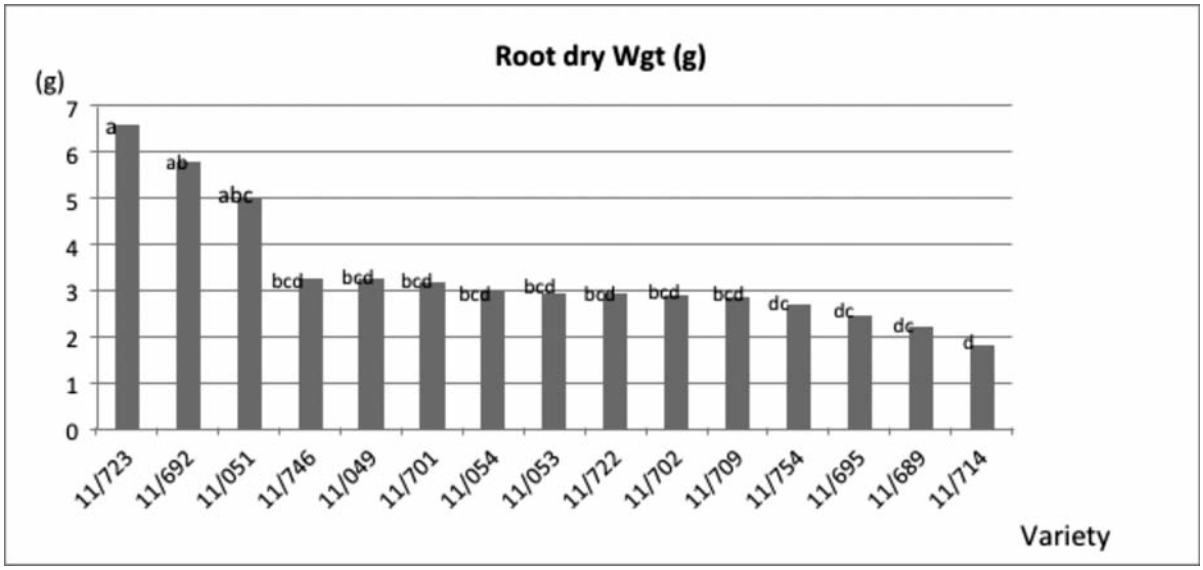


Figure 3:
PCR amplified products of RM 21 primer in 3% agarose gel

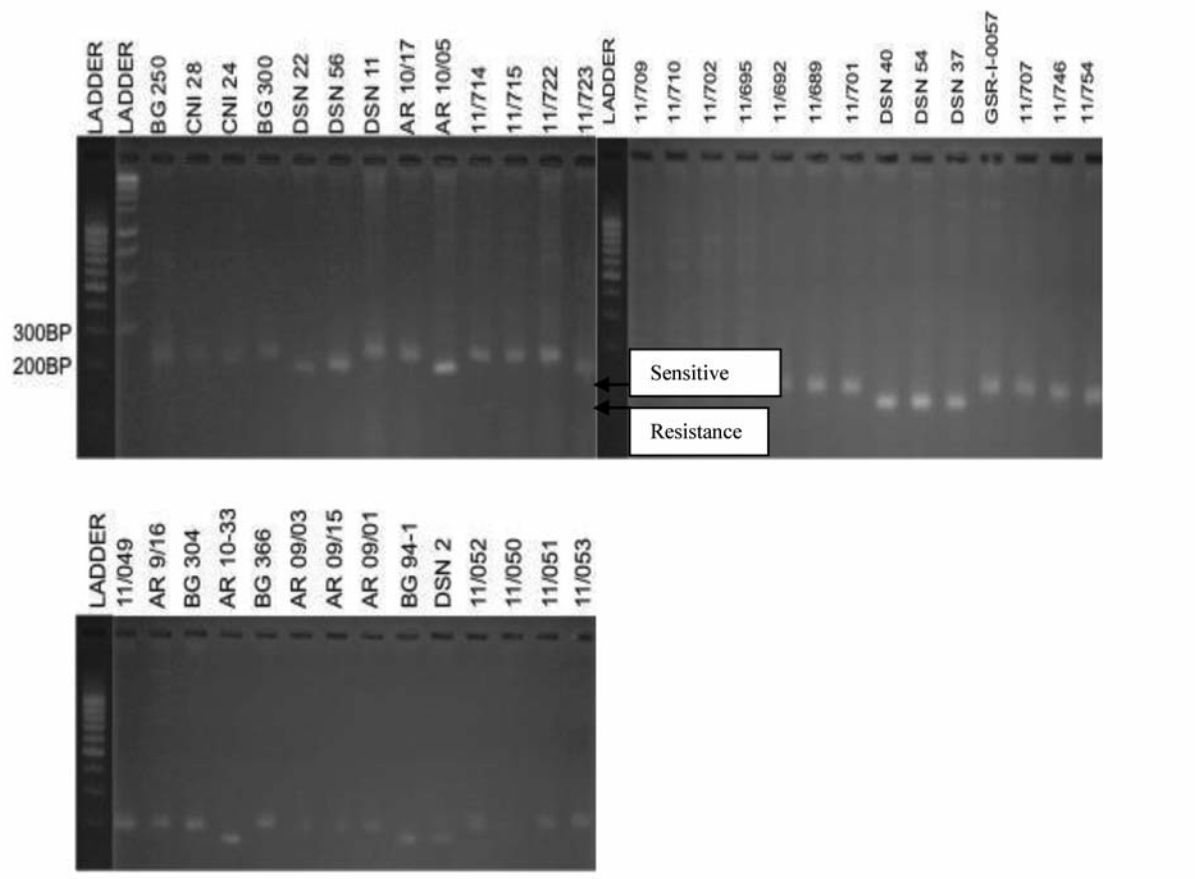
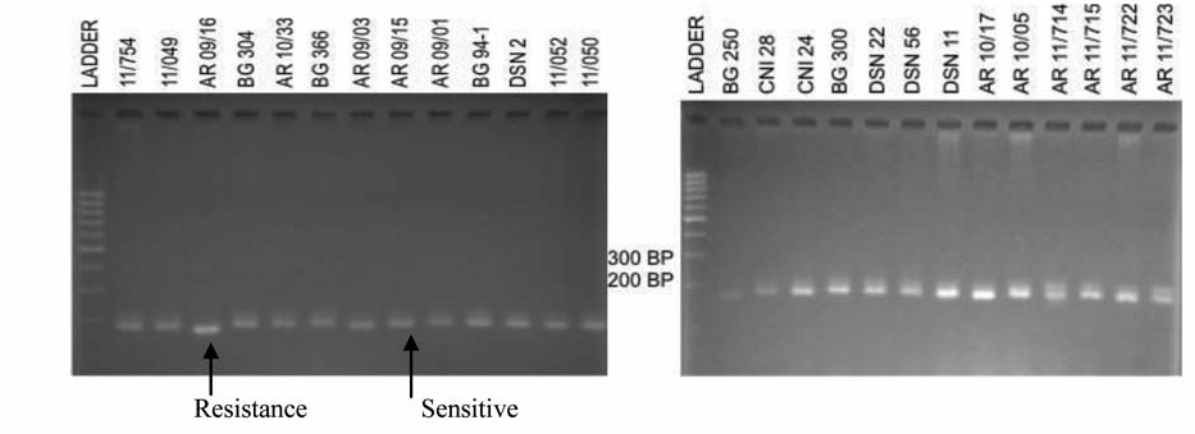


Figure 4:
PCR amplified products of RM 208 primer in 3% agarose gel.



Molecular Screening

Among all tested primers only RM 21 & RM 208 showed polymorphism.

According to the results of PCR amplification; all lines and varieties can categorize in to two groups as drought resistance and drought sensitive. DSN22, DSN56, AR10/05,IR 83140-B-36-B, DSN 40, DSN 54, DSN 37, AR 10/33, BG 94-1, DSN 2, BG 250, and AR 09/16 showed drought resistance bands with microsatellite markers.

Table 2:
Level of drought resistance in tested lines and varieties

Drought character	Varieties/ Lines
Drought resistance	DSN22, DSN56, AR10/05,IR 83140-B-36-B, DSN 40, DSN 54, DSN 37, AR 10/33, BG 94-1, DSN 2, BG 250, AR 09/16
Moderately resistance	IR 83142-B-21-B, HHZ 5-T3-SAL3-DT1, HHZ 11-Y11-Y3-DT1, HHZ 17-DT 6-SAL3-DT1, HHZ 5 SAL 10-DT1-DT1, AR 10/33 Bg 304
Drought sensitive	IR83140-B-11-B, IR 83140-B-28-B, IR 83140-B-32-B,IR 83141-B-17-B, IR 83141-B-18-B, IR 83142-B-19-B, IR 83142-B-20-B,IR 83142-B-21-B, IR 83142-B-49-B, IR 83142-B-57-B, IR 83142-B-60-B,IR 83142-B-61-B, IR 83142-B-79-B, IR 83142-B-7-B-B, IR 83142-B-8-B-B, HHZ 12-Y4-Y3-Y1, HHZ 15-SUB-Y3-Y1,HHZ 5 SAL 10-DT1-DTI, HHZ 5 SAL 10-DT2-DTI, HHZ 5-T3-SAL3-DT1, HHZ 9-DT 7- SAL 2-DT1, HHZ 11-Y11-Y3-DT1, HHZ 17-DT 6-SAL3-DT1,Bg 300, Bg 304, Bg 366, CNI 24, CNI 28,GSR-I-0057.

Conclusion

Results of the morphological and molecular screening revealed that, eight rice varieties and four rice lines are resistant to the water stress in Sri Lankan condition. And many number of rice lines and varieties showed drought sensitive characteristics. So further experiments should be carried out with more markers and should move to a QTL analysis to find the highest QTL value having varieties/lines to find the genomic regions which are responsible for traits.

References

Hanamaratti, N.G., (2007). Identification of QTLs for physiological and productivity traits under drought stress and stability analysis in upland rice (*Oryza sativa* L.), University of Agriculture Sciences, Dharwad, Karnataka State, India.

Kamoshita,A., Rdrigueo, R., Yamushi, J., (2008). Genotypic variation in response of rain fed lowland rice to drought resistance, *Plant production science*, vol.7, pp. 406-420.

Ahmdi, N., Courtois, B., Khowaj, F., Rice, A., Hemlin, C., (2007). Meta –analysis of QTLs involved in rice root development using a QTL database, *proceeding the international symposium on root biology & MAS strategies for drought resistance USA*, sep. 24-29, 2007, IRRI, Philippines.

Fukai, S., (1995). Development of drought-resistant cultivars using physio-morphological traits in rice. *Field Crops Res*. vol. 40, pp.67–86.

Saghai-Maroof, MA., Soliman, KM., Jorgensen, RA., Allard, RW., (1984). Ribosomal DNA spacer-length polymorphisms in barley-mendelian inheritance, chromosomal location, and populationdynamics. *Proc Natl Acad Sci USA*,vol. 81,pp.8014–8018.

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Contribution of *OsDREB1A* and *OsDREB1B* and their putative downstream genes *OsAOX1a* and *OsAOX1b* for cold tolerance in parental cultivars

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Abstract: Based on the trait differences between *Japonica* rice cultivar *Hyogo-Kithanishiki* and *Indica* rice cultivar *Hokuriku*, inbred lines segregating the traits were produced crossing the two parents. One hundred and sixty two inbred lines of F_6 generation derived from the above cross were assessed for cold stress tolerance at 4°C followed by 5 day recovery period. The same population was evaluated for drought and salinity tolerance. *Hygo-Kithanishiki Japonica* parent performed better under each stress compared to *Hokuriku Indica* parent. Expression levels of rice transcription factor genes *OsDREB1A* and *OsDREB1B* and their putative downstream genes *OsAOX1a* and *OsAOX1b* were studied by RT-PCR analysis in parental rice cultivars. Expressions of these genes were examined in parental rice cultivars subjected to salinity, drought, cold and ABA treatment to understand the contribution of transcription factor genes *OsDREB1A*, *OsDREB1B* and stressed inducible genes *OsAOX1a* and *OsAOX1b* for the difference of abiotic stress tolerance levels in two parental rice cultivars *Hyogo-Kithanishiki* and *Hokuriku*.

Introduction

Cold damage is one of the greatest problems in subtropical regions and tropical high-elevated regions in rice growing countries. Cold stress may cause seedling injuries, delayed heading, and also yield reduction due to reduced spikelet fertility (Satake, 1989). Cold stress at the booting stage arrests the microspore development and under severe conditions cause complete yield loss. Importantly, this sterility-inducing cold damage is unrecoverable. Yield loss caused by low temperature is world-wide problem in rice production (Liang, 1998).

Though some *Japonica* rice cultivars show moderate levels of cold tolerance, almost all *Indica* rice cultivars are highly cold susceptible. The *Indica* germplasm has many desirable alleles for improving agronomic characters including grain quality (Andaya, 2003). The most efficient way for the development of cold tolerant *Indica* rice cultivars is to introduce genetic cold tolerance systems into *Indica* rice cultivars from cold-tolerant *japonica* rice cultivars.

Genes induced by cold stress in rice

During cold acclimation significant metabolic and morphological changes occur within the vegetative and reproductive tissues of plants such as leaf structure, membrane composition, protein composition, and sugars and poly amine accumulation (Viswanathan, 2003). Physio-chemical changes may occur in ion exchange channel and in enzyme activities.

Cold responsive genes are differently and/or indirectly related to changes in the tissues during cold acclimation. According to the roles they play, such genes could be categorized into the following gene families; *LEA* - Late embryogenesis abundant protein, *RAB* - Response to abscisic acid, *COR* - Cold responsive, *LTR* - Low temperature tolerance, *DHN* – Dehydrin protein.

Different abiotic stresses may cause general and specific stresses on plants. For example, drought limits plant production due to photosynthetic decline, while salinity stress causes constraint on plant physiological processes and decreases nutrient uptake efficiency. Salinity causes, physiological drought and ion toxicity (Zhu, 2002).

Chilling and freezing temperatures can cause osmotic stress other than its direct effect on metabolism (Thomashow, 1999). During freezing of plant tissues, ice forms first in the extra-cellular compartment, reducing its water potential and leading to loss of water from the cells by osmosis. Thus, freezing stress is indirectly related to dehydration. In plants, stress responsive regulation of gene expression also involves both unique and common changes in transcript levels of the already mentioned cold responsive genes (Shinozaki and Yamaguchi-Shinozaki, 2000). Based on these general phenomena, it is logical to expect that plants have multiple stress perception and signal transduction pathways.

Plants subjected to low but non-freezing temperatures acquire tolerance to freezing temperatures, the process of which is called as cold acclimation (Thomashow, 1999). *COR* genes induced by cold, dehydration, and ABA were shown to be critical in plants for both chilling tolerance and cold acclimation (Thomashow, 1999). Promoters of the *COR* genes contain dehydration responsive elements (*DRE*), C-repeats (*CRT*) and sometimes abscisic acid (ABA) responsive elements (*ABRE*) (Yamguchi-Shinozaki and Shinozaki, 1994). Expression of *COR* genes is controlled by both ABA- dependent and – independent pathways (Shinozaki and Yamaguchi-Shinozaki, 2000). A family of transcription factor genes including C-Repeat binding factor (*CBF*s), or dehydration responsive element binding factor (*DREB*s) control ABA- independent expression of *COR* genes (Yamguchi-Shinozaki and Shinozaki, 1994). The *CBF/DREB* genes are cold stress responsive and their expression precedes induction of expression of downstream genes with the *cis*- elements (Thomashow, 1999)

OsDREB1A and *OsDREB1B*

To survive under stress conditions, rice plants respond to stresses at both molecular and cellular levels and their response differ among different cultivars. A number of genes have been found to be induced by different stresses (Shinozaki and Yamaguchi-shinozaki, 1994, 2000; Thomashow, 1999) in monocot plants including rice. The cold-responsive *CBF/DREB1 AP2* proteins with *AP2* domains are the most well characterized major group of transcription factors regulating the expression of cold-responsive *COR* genes (Seki *et al*., 2001; Fowler and Thomashow, 2002). The dehydration responsive element (*DRE*) was identified as a promoter element, which regulates gene expression in response to drought, high-salt, and cold stresses (Yamaguchi-shinozaki and Shinozaki, 1994). *OsDREB1A* (*CBF3*), *OsDREB1B* (*CBF1*), *OsDREB1C*, *OsDREB1D* and *OsDREB2A* were identified in rice as stress inducible proteins which show homology to *DREB* proteins in *Arabidopsis* (Joseph *et al.,* 2003).

OsDREB1A and *2A* proteins bind to *DRE* and activate transcription of the downstream genes driven by *DRE* in rice. Over-expression of the *DREB1A* cDNA under control of Caulliflower Mosaic Virus (CamV) 35S promoter or the stress inducible *rd29A* promoter conferred strong tolerance to drought, high-salt, and freezing stresses on transgenic *Arabidopsis* (Gilmour *et. al*., 2000; Jaglo-Ottosen *et. al*., 1998, Kazuga *et. al*. 1999; Liu *et. al*., 1998).

Abscisic acid (ABA) is produced under environmental stresses and play important roles in the tolerance of plants to drought, high-salt, and low temperature (Bray, 1997; Ingram, 1996; Jaglo-Ottosan, 1998; Thomashow, 1999). Analysis of the expression of stress-inducible genes in *Arabidopsis* have indicated that at least two ABA-independent signal pathways function in the induction of stress-inducible genes such as *rd29A* (Sakuma *et al.,* 2002). The *cis*-acting element DRE in the promoter region of *rd29A* is responsible for dehydration-, high- salt-, and low-temperature-induced expression.

OsAOX1a and *OsAOX1b*

Growth at low temperature can lead to the increased respiration in cells through the activation of the alternative oxidase (*AOX*) pathway (Jarmuszkiewicz *et al.,* 2001). Jarmuszkiewicz *et al.,* demonstrated this by growing amoeba cells at a low temperature condition and measuring the *AOX* protein amount in the cells. In amoeba the level of *AOX* protein in mitochondria was up-regulated, on average, 1.7- fold under cold stress. A new *AOX* homologous gene *OSIM* in rice was found, which responded to salt stress (Kong *et al.* 2001). The *AOX* activity is up-regulated by a variety of stresses, including chilling and inhibition of cytochrome chain activity or inhibition of amino acid synthesis (Vernlerberghe and Mcintosh 1997). *AOX* is a cyanide insensitive terminal oxidase found in the inner membrane of a variety of organisms and is best characterized in plants. It oxidizes ubiquinol directly and thus bypasses complex III and IV of the cytochrome chain (Millar and Day 1997). Expression of *AOX1a* gene was reported to be induced by treatment with an inhibitor NaN_3, in rice (Saika *et. al*. 2004).

Transcript levels of the *AOX1a* and *AOX1b* genes of rice were studied under various stresses by Ohtsu *et al.,* (2002) and they reported that low temperature, high salt and drought conditions up- regulated the gene expression. Identification of two closely linked QTLs, *qCtb1* and *qCtb2* for cold tolerance at the booting stage in rice was reported by Saito *et.al.* (2001). Abe *et. al*. (2002) found a single nucleotide polymorphism in an *AOX* gene linked to *Ctb2* locus and suggested that *AOX* is related to cold tolerance in rice.

In the present study, inbred line population derived from *Japonica* rice cultivar *Hygo-Kithanishiki* and *Indica* rice cultivar *Hokuriku* were evaluated for cold tolerance. Evaluation protocol was developed using parental rice cultivars and the developed protocol was applied to evaluate the inbred line population (Ranawake *et al.* 2011). Stress responsive genes *OsDREB1A, OsDREB1B, OsAOX1a, OsAOX1b* were used for the gene expression analysis by RT-PCR (Reverse transcriptase polymerase chain reaction) in two parents subjected to cold, drought, salinity stresses and ABA treatment. The objective of the study was to understand the contribution of these genes in cold, salinity and drought stress tolerance in parental rice cultivars.

Materials and Methods

Plant material: A cold tolerant *Japonica* rice cultivar, "*Hyogo-Kitanishiki*" (abbreviated as HGKN), and a cold susceptible *Indica* cultivar, "*Hokuriku* (Yume-Toiro)" (HOK) (Misawa *et al.,* 2000). HOK was bred from a cross between a Korean cultivar "*Milyang 21*" and an IRRI line "IR-2061-214-31" in *Hokuriku* Agricultural Experimental Station, Japan.

Bioassay conditions

Bioassay for cold tolerance was carried out using 2-week-old seedlings. Seed surface sterilization was done by dipping seeds in 70% ethyl alcohol solution for 1 minute and then dipping washed seeds in 1 NaOCl solution for 1 hour. Finally seeds were washed out by autoclaved distilled water. Breakage of dormancy and acceleration of uniform seed germination were performed by keeping surface sterilized seeds at 30 °C for 6 days in distilled water. Imbibed seeds were planted in trays (24 cm X 24 cm) in soil and kept at 25 °C for 7 days under 16 hour photoperiod (normal growth conditions). To minimize the environmental effects, the same growth chamber was used for all the RILs for normal growth conditions and for cold treatments. From each RIL, 20 germinated seeds were planted according to the randomized complete block design with 4 replicates and with 5 plants per replicate. Plants were watered daily and 0.001 % Hyponex (N:P:K=5:10:5 by volume) solution was applied on the every 4th, 11th, and on 20th day after planting.

Plants were subjected to 3-day low temperature treatment at 4 °C under the same light intensity and photoperiod first cold treatment on the 7th day after the planting. After the three-day-cold treatment, plants were returned to the normal growth conditions and kept for 5 days for the recovery. On the 5th day, the levels of cold tolerance were recorded on an arbitrary five-point scale (first rating), where 1- whole seedling is completely withered, 2- leaves are withered but stem remains in green, 3 - only the stem and third leaf (or

one leaf) remain in green, 4 - stem and two leaves remain in green, and 5 - normal growth with all the leaves in green.

HGKN showed an average rating of 4.4, while that of HOK was 1.5. Two RILs were transgressive segregants showing higher levels of cold tolerance than the cold tolerant parent HGKN.

Parental lines were germinated at 30°C for 5 days and seedlings were planted in commercially available soil. Plants were kept at 25°C for 15 days under 16 hour photoperiod. Plants were then subjected to different stress conditions either by keeping them at 4°C for 1 day (cold treatment), applying 200 mM ABA on the leaves and keeping at 25°C for 20 minutes (ABA treatment), dipping plants in 400 mM NaCl solution after washing out soil in the root system and keeping them at 25°C for 1 hour (NaCl treatment), or air dried on dry filter papers at 25°C after washing out soil in the root system (dehydration treatment).

Gene expression analysis

Leaves of seedlings were collected, frozen with liquid nitrogen and were subjected to RNA extraction using guianidine thiocyanate. The amount of transcripts was determined by RT-PCR analysis using a first strand cDNA synthesis kit (TOYOBO, Osaka, Japan). The total template RNA samples for the cDNA synthesis were treated with DNaseI to remove contaminated DNA. RT-PCR was performed with specific primers, which were designed and synthesized by Invitro Lifetech Oriental (Nacalai). Primer information is shown in Table 1. For each sample 4 µl c-DNA template was added to 16 µl reaction mixture containing 1 µl each of forward and reverse primers, 2 µl 10 x buffer, 0.8 µl $MgCl_2$, 1 µl dNTPs, 10 µl Q water and 0.2 µl rTaq Polymerase to make a 20 µl PCR mixture. RT-PCR was carried out by amplification with 22 cycles under conditions described in Table 1 using a thermal cycler, Gene Amp PCR System 9700 (Applied Biosystem). For each sample, 4 µl c-DNA template was added to 16 µl reaction mixture. Rice actin gene was used as a control. RT-PCR products were resolved on 1.2 % or 2.0 % Agarose gel, stained with Ethidium Bromide, and pictures of images were taken under UV light.

Results and Discussion

The plants were evaluated using a 5-point rating scale at 25˚C after the cold treatment. By the first day of the recovery period only five plants out of 40 plants were recovered in the cold susceptible *Hokuriku*, while the number of survived plants was 40 in the cold tolerant HGKN. On the fourth day of the recovery period, all the plants died in *Hokuriku* but in HGKN 32 plants were recovered. By the fifth day of the recovery period, 30 plants in HGKN were recovered and started normal growth under the normal growth conditions, and after that no more plants died during the following three days of the recovery period. The same sets of plants were subjected to the second-3 day cold treatment. HGKN plants could survive the second cold treatment, whereas all the plants of *Hokuriku* were died. A new five-point rating scale was applied to evaluate the level of cold tolerance after the second cold treatment.

Gene expression analysis

To see the effect of two transcription factor genes *OsDREB1A* and *OsDREB1B* and their putative downstream gene *AOX1a* and *AOX1,* RT-PCR analysis was done for the cold stress parental rice cultivars.

Both of *OsDREB1A* and *OsDREB1B* were induced in both cold tolerant *Hyogo-Kithanishiki* and in cold susceptible *Hokuriku* under all the stress conditions and the levels of expression of the genes were differed under different stresses.

OsAOX1a and *OsAOX1b* were induced under different stress conditions including cold stress.

The results showed that *OsDREB1A* and *OsDREB1B* were expressed in significantly higher level under cold stress in both cultivars (Fig. 1) compared that of in control plants. At the seedling stage of rice, 3-day cold stress induced the expression of these transcription factor genes in both cold tolerant and in cold susceptible parents while the induction levels of the both genes were much higher in cold tolerant cultivar HGKN. The induction level of *OsDREB1A* and *OsDREB1B* by ABA was similar to those by cold stress. These transcription factor genes also appeared to be

induced by other stresses at least by NaCl. The mode and role of expression of these genes have to be further studied.

RT-PCR results of the present study suggested that *OsAOX1a* and *OsAOX1b* were induced in both HGKN and HOK not only by the cold stress but also by the other stresses; ABA, Dehydration and NaCl (Fig. 2). Transcript levels of alternative oxidase genes *AOX1a* and *AOX1b* of rice have been studied under various stresses (Ohtsu *et al.* 2002). They reported that low temperature, high salt and drought conditions up-regulate the genes. Abe *et al.* (2002) also found a single nucleotide polymorphism in an *AOX* gene linked to Ctb2 (cold tolerant booting stage) QTL and suggested that *AOX* is related to cold tolerance. In the present study the transcripts of both genes *OsAOX1a* and *OSAOX1b* could be seen in the control plants also, but the expression levels in the stressed plants compare to the control plants appeared to be higher in HGKN cold tolerant line at least for *OsAOX1b* (Fig.2). In case of *OsAOX1a* the expression levels appeared to be higher both in the control and the cold stressed plants of HGKN than in HOK.

Expression of transcription factor genes *OsDREB1A*, *OsDREB1B* and stressed inducible genes *OsAOX1a* and *OsAOX1b* are responsible for the difference of cold tolerance levels in two parental rice cultivars HGKN and *Hokuriku*.

Table 1 :
Primers used for gene expression analysis

Gene	Forward primer	Reverse primer
OsDREB1A	5' UCGAGCAGAGCAAAUACAGU 3'	5' AUCGGAAGCCAGAAAAGAGA 3'
OsDREB1B	5' AUGGAGGUGGAGGAGGCGGC 3'	5' GUCCUCCCACCACGCUCCGG 3'
OsAOX1a	5' GAT GTT TGT CTA CTG CCG AGG ATT T 3'	5' ATG TTAG TAT ATA TAA CTC AGC TGC C 3'
OsAOX1b	5' TCA TCA TTC ATC AAC GGG CGA TGC 3'	5' TGT GC A CGG GTC AGC CCA CGG CCA 3'

OsDREB PCR programme: 94°C 5minutes, 94°C 30 seconds, 55°C 30 seconds, 72°C 30 seconds
OsAOX PCR programme: 94°C 5minutes, 94°C 1 minute 55°C 1 minute 72°C 30 seconds

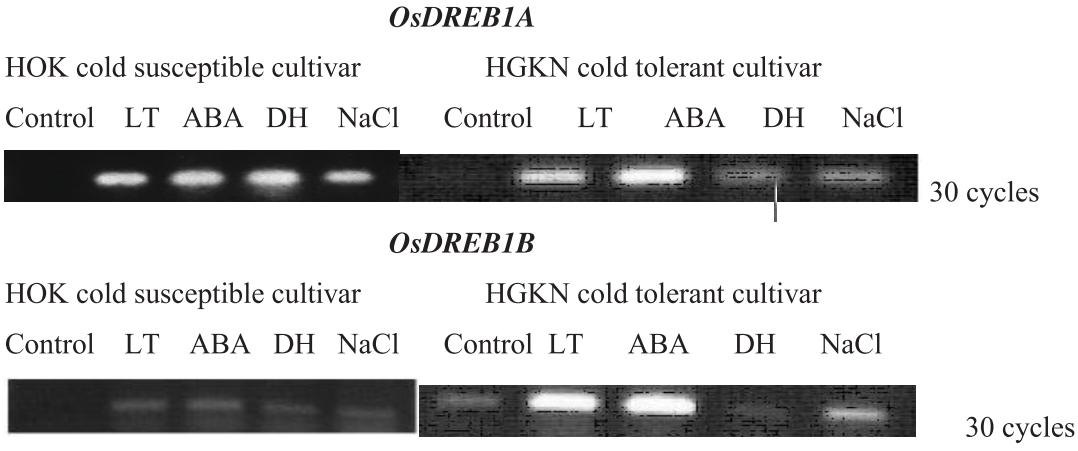
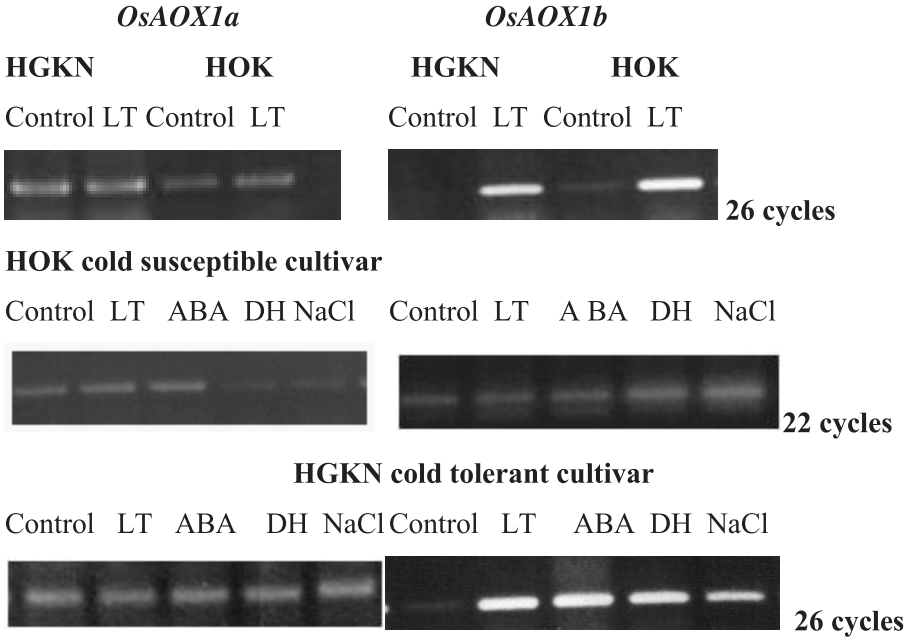


Fig. 2: RT-PCR analysis of the transcript levels of the rice alternative oxidase genes *OsAOX1a* and *OsAOX1b* under stress conditions. LT, cold treatment at 4°C for three days; ABA, 200mM exogenous ABA for 20 minutes; DH, dehydration at 25°C for 6 hours; NaCl, high salt in 400mM NaCl solution for 6 hours; HGKN: *Hyogo-Kithanishiki* cold tolerant cultivar, HOK: *Hokuriku* cold susceptible cultivar.



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References

Abe F, Saito K, Miura K, Toriyama K, 2002 A single nucleotide polymorphism in the alternative oxidase gene among rice varieties differing in low temperature tolerance. FEBS lett. 527: 181-185

Andaya VC, Mackill DJ, 2003 QTLS conferring cold tolerance at the booting stage of rice using recombinant inbred lines from a *japonica* X *indica* cross. Theor Appl Genet. 106 (6); 1084 – 1090

Bray EA, 1997 Plant responses to water deficit. Trends Plant Sci. 2; 48–54.

Flower S, MF Thomashow 2002 Arabidopsis transcriptome profiling indicates that multiple regulatory pathways are activated during cold acclimation in addition to the CBF cold response pathway. Plant Cell. 14(8): 1675-90.

Gilmour SJ, Sebol AM, Salazar MP, Everard JD, and Thomashow MF, 2000 Over-expression of Arabidopsis CBF3 transcriptional activator mimics multiple biochemical changes associated with cold acclimation. Plant physiol. (124): 1854-1865

Ingram J, and Bartel D, 1996 The molecular basis of dehydration tolerance in plants. Annu. Rev. Plant Physiol. Plant Mol. Biol. (47): 377–403

Jaglo-Ottesen KR, Gilmour SJ, Zarka DG, Schabenberger O, And Thomoshow MF, 1998 Arabidopsis CBF1 over-expression induces COR genes and enhances freezing tolerance. Science. (280): 104 – 106

Jarmuszkiewicz W, Frczyk O, Hryniewiecka L, 2001 Effect of growth at low temperature on the alternative pathway respiration *in Acanthamoeba castellanii* mitochondria. Acto Biochinica Polonica, 48(3): 729–737

Joseph GD, Sakuma Y, Ito I, Kasuga M, Dubouzet EG, Miura S, Suki M, Shinozak, Y, and Shinozaki K, 2003 *OsDREB* genes in rice, (*Oryza sativa* L.,) encode transcription activators that function in drought-, high-salt- and cold-responsive gene expression. The Plant Journal. (33): 751-763

Kazuga M, Liu Q, Miura S, Yamaguchi-Shinozaki K, And Shinozaki K, 1999 Improving plant drought, salt and freezing tolerance by gene transfer of a single stress-inducible-transcription factor. Nat. Botechnol.(17): 287 – 291.

Kong J, Gong JM, Zhang ZG, Zhang JS, Chen SY 2003 A new AOX homologous gene OsIM1 from rice (*Oryza sativa* L.) with an alternative splicing mechanism under salt stress. Theor Appl Genet (107): 326–331

Liang Y, 1998 Current status of rice cold tolerance researches in Asia. Journal of Yunana Agr. Uni. 13(1): 35

Liu Q, Kasuga M, Sakuma Y, Abe H, Miura S, Yamaguchi-Shinozaki K, And Shinozaki K, 1998 Two transcription factors *DREB1* and *DREB2* with an *EREBP/AP2* DNA binding domain separates two cellular transduction pathways in drought and low-temperature-responsive genes expression respectively in *Arabidopsis*. Plant cell. 19: 1391 - 1406

Millar AH, Finnegan PM, Whelan J, Drevan JJ, Day D A, 1997 Expression and kinetics of the mitochondrial alternative oxidase in nitrogen fixing nodules of soybean roots. Plant cell and environment. 20: 1273-1282

Ohtsu K, Ito Y, Saika H, Nakazono M, Tsutsumi N, and Hirai A, 2002 ABA – independent expression of rice alternative oxidase genes under environmental stresses. Plant Biotechnology. 19 (3): 187-190

Ranawake AL, Nakamura C, 2011 Cold tolerance of an inbred line population at different growth stages in rice (*Oryza sativa* L.), Tropical Agriculture Research and Extension, 14(2): 25-33

Saika HN, Tsutsumi A, and Nakazono M, 2004 Transcript level of rice alternative oxidase 1a gene increases by treatment with sodium azide, an inhibitor of cytochrome c oxidase. Research notes, Genetics of physiological traits and others, 19

Saito K, Miura K, Nagano K, Hayano-Saito Y, Araki H, Kato A, 2001 Identification of two closely linked quantitative trait loci for cold tolerance on chromosome 4 of rice and their association with anther length. Theor. Appl. Genet. 103: 862 – 868

Sakuma Y, Liu Q, Dubouzet JG, Abe H, Shinozaki K, and Yamaguchi-Shinozaki K, 2002 DNA-binding specificity of the ERF/AP2 domain of Arabidopsis DREBs, transcription factors involved in dehydration- and cold-inducible gene expression. Biochem. Biophys. Res. Commun. 290: 998–1009.

Satake T. 1976 Determination of the most sensitive stage to sterile-type cool injury in rice plants. Plant Cell 14: 1675-1690.

Shinozaki K, and Yamaguchi-Shinizaki K, 1994 Gene expression and signal transduction in water-stress response. Plant physiol. 115: 327-334

Shinozaki K, And Yamaguchi-Shinozaki K, 2000 Molecular response to dehydration and low temperature: differences and cross-talk between two stress signaling pathways. Curr. Opin. Plant. Biol. 3: 217-223

Thomashow MF, 1999 Plant cold acclimation: freezing tolerant gene regulatory mechanisms. Annu. Rev. Plant physiol. Plant mol. Biol. 50: 571-599

Vanlerberghe GC, McIntosh L, 1997 Alternative oxidase: from gene to function. Annu. Rev. Plant. Physiol. Plant Mol. Biol. 48: 703-734

Viswanathan C, Karen S, and Jian-Kang Zhu, 2003 Molecular genetic perspectives on cross-talk and specificity in abiotic stree signalin in plants, Journal of Experimental Botany. 55 (395): 226-236

Yamaguchi-Shinozaki K, and Shinizaki K, 1994 A novel *cis*-acting element in responsiveness to drought, low temperature or high-salt stress. Plant cell. 6: 251-264

Zhu J-K, Liu J, Xiong L, 1998 Genetic analysis of salt tolerance in *Arabidopsis thaliana:* evidence of a critical role for potassium nutrition. Plant Cell. 10: 1181-1192