ISSN 2221-6421 (Print), ISSN 2223-2567 (Online)
Coden: PJSIC6 59(3) 117-174 (2016)

Pakistan Journal of Scientific and Industrial Research

Series B: Biological Sciences

Vol. 59, No.3, November-December, 2016

(for on-line access please visit web-site http://www.pjsir.org)

Published by
Scientific Information Centre
Pakistan Council of Scientific and Industrial Research
Karachi, Pakistan

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Series B: Biological Sciences

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Printed and Published by: PCSIR Scientific Information Centre, PCSIR Laboratories Campus, Shahrah-e-Dr. Salimuzzaman Siddiqui, Karachi-75280, Pakistan.

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Tel: 92-21-99261914-99261916, 99261949-99261917; Fax: 92-21-99261913; Web: http://www.pjsir.org, E-mail: info@pjsir.org

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Contents

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Pak. j. sci. ind. res. Ser. B: biol. sci. 2016 **59**(3) 117-125

Effect of Terminal Drought Stress on Morpho-physiological Traits of Wheat Genotypes

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(received May 05, 2015; revised March 30, 2016; accepted April 15, 2016)

Abstract. Development of wheat varieties with low moisture requirements and their ability to withstand moisture stress may cope-up well with the on-coming peril of drought conditions. Ten wheat genotypes including two new strains, PBGST-3, Hero, Bhittai, Marvi, Inqlab, Sarsabz, Abadgar, Kiran, Khirman and PBGST-4 were sown in split plot design with factorial arrangement in four replications at Experimental Field, Department of Plant Breeding & Genetics, Sindh Agricutlure University, Pakistan during 2012-13. The results revealed that water stress caused significant reductions in all morpho-physiological traits. The genotypes differed significantly for all the yield and physiological traits. The interaction of treatments × genotypes were also significant for all the traits except plant height, productive tillers/plant, grains/spike and harvest index, were non-significant which indicated that cultivars responded variably over the stress treatments suggesting that breeders can select the promising genotypes for both stress and non-stress environments. Among the genotypes evaluated Bhittai, Kiran-95, PBGST-3 and Sarsabz showed good performance as minimum reductions occurred under terminal stress conditions for all the traits studied. Hence, above mentioned genotypes were considered as drought tolerant group. The high positive correlations of physiological traits like chlorophyll content and relative water content with almost all yield traits indicated that these physiological traits could serve as reliable criteria for breeding drought tolerance in wheat. The negative correlations of electrolyte leakage with several important yield traits indicated that though this physiological trait has adverse effect on yield attributes, yet it could reliably be used to distinguish between drought tolerant and susceptible wheat genotypes.

Keywords: drought stress, yield attributes, physiological traits, correlations, wheat genotypes, eleclrolyte leakage

Introduction

Although breeders are continuing to improve the yield potential of wheat, however, progress to achieve increasing wheat yields in drought environments has become more difficult (Jones, 2007). In defining a strategy for wheat breeding under drought tolerance, Rajaram *et al.* (1996) suggested that simultaneous evaluation of germplasm should be carried-out both under near optimum conditions (to utilize high heritability and identify genotypes with high yield potential) and under stress conditions (to preserve alleles for drought tolerance). In wheat, yield is reduced mostly when drought stress occurs during heading or flowering and soft dough stages. Drought stress during maturity resulted in about 10% decrease in yield, while moderate stress during the early vegetative period had essentially no effect on yield (Jatoi *et al*., 2012). Munjal and Dhanda (2016) noted that mean performance of wheat genotypes for grain yield under irrigated conditions was significantly higher than under drought stress conditions. Therefore, physiological and biochemical approaches have a great importance in order to understand the complex responses of plants to water deficiency which could help to develop new varieties rapidly. Development of cultivars with high yield is the main goal in water limited environments but success has been modest due to the varying nature of drought and the complexity of genetic control of plant responses (Mirbahar *et al*., 2009). Various quantitative traits (including morphological and physiological characteristics) have been proposed for the selection of tolerant genotypes to drought stress (Hammad *et al*., 2014). A wide range of putative selection criteria that could be used to increase drought tolerance in plants is available, however, very few examples of success obtained using physiological traits in breeding programmes. A physiological approach would be the most attractive way to develop new wheat varieties, but breeding for specific and sub-optimal environments involve a deeper understanding of yield determining process (Araus

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et al., 2008). Total chlorophyll content and the chlorophyll a/b ratio were found to reduce under water stress conditions. A decrease in this index was faster in drought sensitive than in drought tolerant genotypes (El-Tayeb, 2006). Rong-Hua *et al.* (2006) concluded that chlorophyll content with SPAD units could be considered as a reliable indicator in screening barley genotypes for drought tolerance. Water deficiency was found to reduce the relative water content (RWC) in plant leaves. A high RWC and low excised leaf water loss (RWL) have been suggested as important indicators of water status (Arjenaki *et al*., 2012).

Drought is a serious problem in many parts of the world (Moayedi *et al*., 2011) where wheat, barley and other small-grained cereals are part of the staple diets. Opportunities for marker-assisted selection are also considered. Incorporating specific drought resistance traits in breeding programmes should facilitate more rapid improvement in the drought resistance of wheat and other small-grained cereals (Quarrie *et al*., 1999).

Drought at grain filling stage reduces the cell size and number and results in shriveled grains with small size and reduced weight and early maturity (Gomaa *et al*., 2014). It has been found that under the drought stress conditions, those genotypes that show the highest harvest index and highest yield stability are drought tolerant (Rathore, 2005). The main objectives of present study therefore were: (i) to determine the mean performance of wheat genotypes for water stress tolerance, (ii) to identify drought tolerance indicators based on morpho-physiological traits and (iii) to determine correlations between various morpho-physiological traits under terminal water stress.

Materials and Methods

The field experiment was conducted at Experimental Field of the Department of Plant Breeding and Genetics, Sindh Agriculture University, Tandojam, Sindh, Pakistan so as to screen drought tolerance wheat genotypes during 2012-13 cropping season. The experiment was carried-out in split plot design with two treatments (non-stress and stress at anthesis) in four replications. Ten genotypes viz. PBGST-3, Hero, Bhittai, Marvi, Inqlab, Sarsabz, Abadgar, Kiran, Khirman and PBGST-4 were sown through hand drill. The water regimes were considered as the main factors while wheat genotypes as sub-factor. The irrigation regimes with no stress treatment received frequent irrigations without any water stress (a total of 6 irrigations were applied), while in water stress treatment, stress was imposed at anthesis by with-holding water for 40 days from initiation of anthesis till start of grain formation.

The essential cultural operations were adopted uniformly in all the plots throughout the growing period. Before first irrigation, seedlings were thinned to ensure uniform and reduced plant competition for optimum plant growth and development. All the agronomic practices were done at proper time. Fertilizer at the rate of 125-75 kg N&P/ha, respectively, was applied in the form of Urea and DAP. Full dose of phosphorus with 1/3rd of nitrogen was applied at the time of land preparation while remaining 2/3rd nitrogen were split in three equal doses and applied with first, third and fifth irrigations. Other inputs like herbicides were applied as and when required. All the required cultural practices including dry hoeing, weeding etc. were adopted uniformly in all plots throughout the growing period. Data were collected from ten randomly tagged index plants from each genotype per replication for yield traits like plant height (cm), productive tillers/plant, grains/spike, seed index (1000 grain wt. in g), grain yield (kg/ha) and harvest index (%). The relative water content (RWC%) was determined with formula developed by Schonfeld *et al.* (1988): RWC% = (fresh weight –dry weight) / (turgid weight–dry weight) × 100, chlorophyll content was measured by SPADE meter (SPAD-500 Plus) as relative greenness in arbitrary units and electrolyte leakage(%) was assayed by estimating the ions leaching from the cell wall with the procedure developed by Sairam *et al.* (1998). Plant material (0.3 g) was taken in 10 mL of de-ionized water in two sets. One set was subjected to room temperature (approx. 25 °C) for 4 h and its conductivity (C1) was recorded using a conductivity instrument (LC116, Mettler-Toledo Instruments Co., Ltd, Shanghai, China). The other set was kept in a boiling water bath (100 °C) for 10 min and its conductivity was also recorded (C2). Electrolyte leakage was calculated as:

$$\text{Electrolyte leakage} = [1 - (C1/C2)] \times 100.$$

Statistical analysis. Analysis of variance was carried out according to procedures developed by Gomez and Gomez (1984) whereas, phenotypic correlations were determined according to Raghavrao (1983) by using the following formula:

$$r = \sqrt{\frac{[\Sigma xy-(\Sigma x)(\Sigma y)/n]}{\Sigma x^2 - (\Sigma x)^2/n \times \Sigma y^2 - (\Sigma y)^2/n}}$$

Results and Discussion

Analysis of variance. Mean squares from analysis of variance (Table 1) revealed that water stress caused significant declines in plant height, productive tillers/plant, grains/spike, seed index, harvest index, grain yield (kg/ha), relative water content (RWC %), chlorophyll content (relative greenness) and electrolyte leakage (EL %). Significant differences were also observed among the cultivars for all the yield and physiological traits studied that could help wheat breeders to select the drought tolerant varieties on the basis of one or more morpho-physiological attributes. The mean squares due to treatment × genotype interactions were also significant for all these traits except that plant height, productive tillers/plant, grains/ spike and harvest index were non-significant. The significance of treatment × genotype interactions indicated that varieties performed variably over the stress treatments. These interactions could help wheat breeders to select the promising varieties based on one or more reliable drought tolerant indicators and put them in a breeding programme to develop new drought tolerant breeding material. Similarly, several researchers like Allahverdiyev *et al.* (2015); Baloch *et al.* (2012) and Jatoi *et al.* (2012) reported significant difference in response of wheat varieties to terminal water stress conditions.

Mean performance of wheat cultivars under terminal water stress. ***Plant height (cm).*** Optimum plant height is considered as an important trait for avoiding lodging, thus maximizes harvest index. On an average, water stress caused -5.06 cm reduction in plant height yet, minimum reduction was observed in Sarsabz (-3.00 cm) while maximum in Kiran (-7.37cm) followed by Hero (-6.47) and PBGST-03 (-6.18 cm) (Fig. 1a). The lowest decrease in plant height of later group of varieties indicted their tolerance however, such statement may not hold true where terminal drought is expected having no effect on plant height when it is already attended before stress was imposed. Similar results were noted by Jatoi *et al.* (2012); Khakwani *et al.* (2011) and Mirbahar *et al*. (2009); who observed that water stress significantly reduced the plant height.

Productive tillers/plant. On an average, water stress caused a decline of -1.85 tillers per plant (Fig. 1b). The minimum relative decreases were recorded in Bhittai, Kiran, Sarsabz and Khirman being stress tolerant genotypes. While the prominent reductions were observed in cultivars Abadgar, Hero and Inqlab. The minimum and maximum reductions due to stress in above cultivars for tillers/plant characterised first group as drought tolerant and second as drought susceptible ones. Present results are in accordance with those recorded by Jatoi *et al.* (2011) and Baloch *et al.* (2012)

Grains/spike. The range of seeds set by single spikes was counted as 57.73-84.39 in normal water conditions, while in stress, the range was 51.55-78.91 grains/spike. On an average, water stress caused -5.98 seeds decline in grains/spike. When comparing the cultivars, highest number of grains/spike were set by Bhittai, Hero and PBGST-4 in water stress at anthesis, respectively (Fig. 2a). Usually, water stress at terminal stage causes infertility which results into lower number of grains/spike. Similar results were suggested by Allahverdiyev *et al.* (2015) and Elhafild *et al.* (1998) who demonstrated that drought stress results in reduced pollination and reduces the number of grains/spike.

Table 1. Mean squares from analysis of variance for various morpho-physiological traits of wheat genotypes grown under water stress conditions

Yield traits	Replication D F = 3	Treatment (T) D F = 1	Error (a) D F = 3	Genotypes (G) D F = 9	T × G D F = 9	Error (b) D F = 54
Plant height	28.50	513.08**	3.34	140.04**	3.49ns	6.07
Prod. Tillers/plant	0.62	68.62**	0.66	11.50**	0.33 ns	0.36
Grains/spike	3.64	715.33**	9.33	489.39**	6.15ns	7.44
Seed index	18.49	783.82**	1.40	73.60**	16.02**	3.44
Harvest index	4.56	555.30**	3.82	35.20**	1.87 ns	9.32
Grain yield (kg/ha)	499	8156206**	1487	1305922**	138968**	3395
Physiological traits						
Relative water content	1.00	26938.90**	1.40	144.70**	59.10**	1.80
Chlorophyll content	4.32	1496.80**	5.90	100.38**	3.64*	3.80
Electrolyte leakage	67.75	8181.01**	6.88	98.67**	51.40**	7.05

**,* = significant at 1 and 5% probability levels; DF = degrees of freedom; ns = non significant.

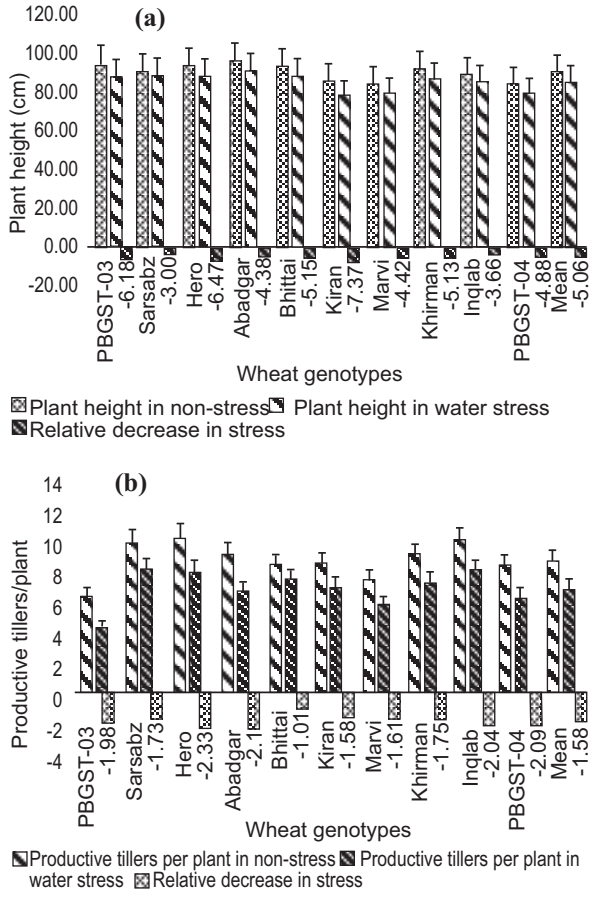

Fig. 1(a-b). Mean performance for plant height (a) and number of productive tillers plant-1 (b) of wheat genotypes grown under non-stress and water stress at anthesis.

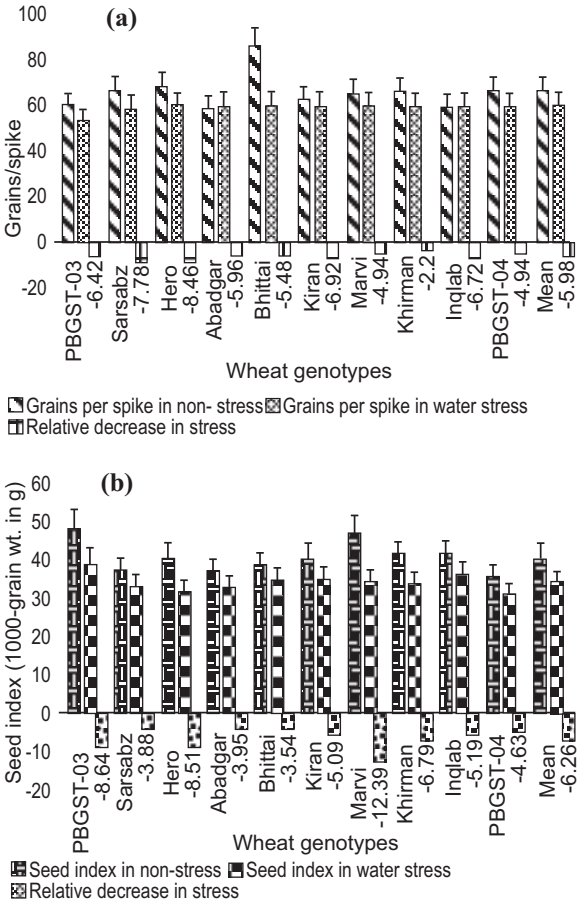

Fig. 2(a-b). Mean performance for grains/spike (a) and seed index (b) of wheat genotypes grown under non-stress and water stress at anthesis.

Seed index (1000-grain wt. in g). The average seed index in non-stress was 40.04 g while in stress conditions was 33.78g, thus on an average, water stress caused -6.26 g reduction in thousand grain weight (Fig. 2b). The little declines in seed index due to terminal stress however were recorded in cvs. Bhittai, Sarsabz, Abadgar and PBGST-4, whereas, sharper reductions were noted in cultivars Marvi, PBGST-03 and Hero. Based on these results, the first group of cultivars was considered as drought tolerant and second group as drought susceptible. During grain formation, water stress reduces transport of assimilates to the grains resulting smaller seeds, consequently lower seed index. Plaut *et al.* (2004) reported that 1,000 kernel weight and weight of kernels per spike were more severely decreased by water deficit i.e., the rate of dry matter accumulation and number of kernels were considerably decreased due to water deficit. Jatoi *et al.* (2012) also recorded similar results.

Harvest index (%). One useful approach to increase wheat productivity is to split yield into biomass at maturity and harvest index (HI). Most yield potential progress in wheat has been associated with increased HI. It is often stated that progress in HI is exhausted because values are approaching the limits of 60%, hence the focus should be on biomass rather than on HI. In present study, the average HI due to water stress dropped by -5.27%, however, this decline was smaller in some of the cultivars such as Sarsabz, PBGST-4 and PBGST-3 while sharper reductions occurred in Marvi , Khirman and Abadgar as shown in Fig. 3a. Thus, these two groups of cultivars could be considered as highly drought tolerant and highly susceptible ones, respectively, yet the remaining cultivars fall in moderately tolerant. These results are in conformity with those of Jatoi *et al.* (2011), who reported that the average HI dropped due to water stress however, this decline was smaller in tolerant cultivars.

Grain yield (kg/ha). Grain yield (kg/ha) is the ultimate result of all physiological and agronomical responses of varieties to drought stress conditions. The average decline due to terminal water stress was recorded as -638.60 kg/ha (Fig. 3b). The higher grain yield in kg/ha was produced by PBGST-03, PBGST-04, Bhittai and Sarsabz in water stress imposed at anthesis. Thus, these cultivars sustained the water stress by showing drought tolerance against other cultivars under evaluation. Drought during grain filling could be limiting the rate and duration of filling processes, causing small grain size, earlier physiological maturity, reduce number of grains, low grain weight and grain yield of wheat (Gupta *et al*., 2001). Allahverdiyev *et al.* (2015) reported that drought led to decrease in yield and yield components of wheat genotypes. Munjal and Dhanda (2016) also reported that about 50% reduction was observed in grain yield under stress condition.

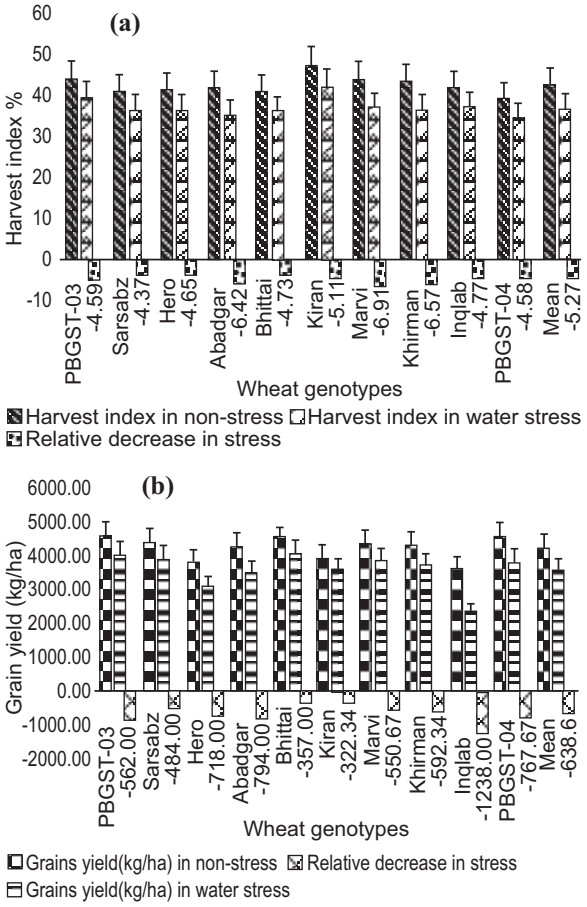

Fig 3(a-b). Mean performance for a) harvest index and (b) grain yield kg/ha of wheat genotypes grown under non-stress and water stress at anthesis.

Relative water content (%). Relative water content (RWC %) is very essential criteria of water stress in wheat leaves. The average reduction of -36.70% in RWC % was noticed due to water stress (Fig. 4a). The top three cultivars having higher RWC% in stress conditions were Bhittai, Abadgar and Marvi, while, the lowest RWC% was observed in PBGST-04, Hero, Sarsabz and Khirman. These results indicated that first group of cultivars was drought tolerant and second being drought susceptible, yet the remaining cultivars were moderately drought tolerant. These results indicated that first group of cultivars was drought tolerant and second being drought susceptible, yet the remaining cultivars were moderately drought tolerant. These findings were similar to those noted by Gunes *et al.,* (2008) who reported that water deficient was found to reduce the relative water content (RWC%) in plant leaves.

A high RWC% and low excised leaf water loss (RWL) have been suggested as important indicators for selection of drought tolerant genotypes. Schonfeld *et al.* (1988) also observed a decline in the amount of RWC% of wheat due to drought stress and reported the highest RWC% in the tolerant genotype.

Chlorophyll content (relative greenness, RG). Leaf chlorophyll increases the photosynthetic activity, hence contributes toward more grain yield. The chlorophyll content decreased in water stress treatment with an average of -8.65 RG (Fig. 4b). The maximum chlorophyll content was recorded in Inqlab followed by Abadgar and Marvi in non-stress whereas, in stress cultivars Abadgar gave highest chlorophyll content (49.26 RG) followed by Inqlab and Bhittai (48.88 RG). High chlorophyll content is a desirable characteristic because it indicates a lower degree of photo-inhibition of photo-synthetic apparatus, therefore, reducing carbohydrate losses for grain growth (Farquhar *et al.*, 1989). These findings are in conformity with those obtained by Iturbe *et al.* (1998) who reported that water stress condition caused reduction in chlorophyll content. Decrease in the chlorophyll content under drought stress was also observed by Sayar *et al.* (2008) in wheat.

Electrolyte leakage (%). Cell membranes are one of the first targets of many plant stresses and it is generally accepted that the maintenance of their integrity and stability under water stress conditions is a major component of drought tolerance in plants. The degree of cell membrane injury induced by water stress may be easily estimated through measurements of electrolyte leakage from the cells (Bajji *et al*., 2001). Less electrolyte leakage

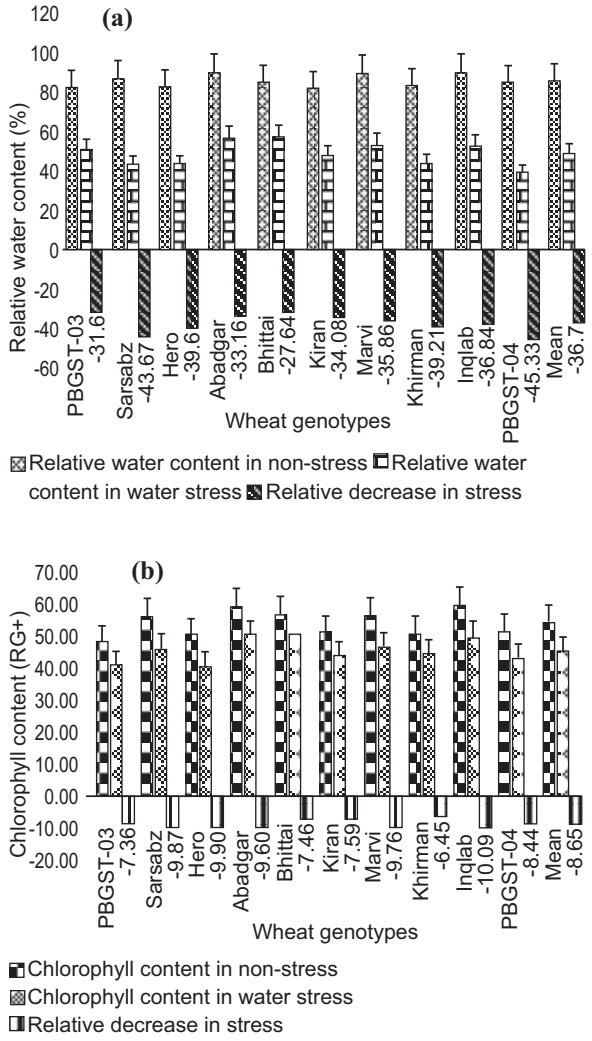

Fig 4(a-b). Mean performance for a) relative water content and b) chlorophyll content of wheat genotypes grown under non-stress and water stress at anthesis.

(EL %) is an important indicator of water stress tolerance in leaves under drought conditions. In non-stress, the EL% varied from 9.25-13.75% whereas in stress, the range was 22.00 to 39.00%. However, on an average, cell membrane leakage of 20.23% was noticed due to water stress (Fig. 5). The top three cultivars with lower percent of membrane leakage in stress conditions were, Bhittai, Kiran and Abadgar, while the highest leakage percentage was marked in PBGST-04, Inqlab, Marvi and Sarsabz. These results indicated that first group of cultivars was drought tolerant and second being drought susceptible, yet the remaining cultivars were moderately drought tolerant. Similar to our results (Bajji *et al.* 2001) also noted that injury index of drought sensitive cultivar Kabir-1 exhibited highest values as compared with the drought resistant cultivars which gave lower injury percentage. Sayar *et al.* (2008) noted that electrolyte leakage reached at 21 and 11% after 2 h in drought susceptible and tolerant wheat cultivars.

Correlations between yield and physiological traits. Though most of the yield traits were significantly correlated with each other, yet the correlation coefficient (r) was at lower side to most part (Table 2). The plant height, productive tillers/plant were significantly but moderately associated with all the yield traits under study. The high correlation (r = 0.45**) however was recorded between productive tiller/plant and grain yield/plant. Among the yield traits, the maximum correlations nevertheless was obtained between grains/spike and grain yield/plant (r = 0.63**) and seed index with harvest index (r=0.62**). The most valuable correlations nonetheless were noted among grains/spike, seed index, harvest index and seed yield/plant and kg/ha. In drought stress and non-stress conditions, spike length had positive and significant correlation with number of grains/spike. Azadi *et al.* (2009) also reported positive and significant correlation between spike length with number of spikelets/spike, spike weight and number of grains/spike in wheat under drought stress condition. Golparvar *et al.* (2006) investigated some bread wheat cultivars in two conditions of drought stress and non-stress and they observed positive and significant correlation between spike length with number of grains/spike.

The correlation coefficients between yield and physiological traits were also recorded (Table 2) which

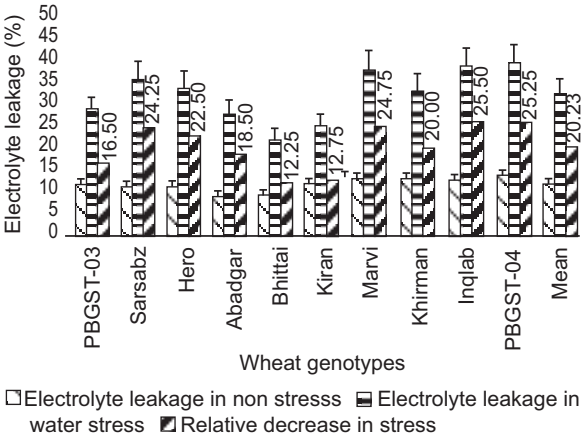

Fig 5. Mean performance for electrolyte leakage of wheat genotypes grown under non-stress and water stress at anthesis.

indicated that traits like plant height, tillers/plant, grains/spike, seed index and harvest index were negatively but significantly associated with electrolyte leakage. Other physiological traits like relative water content was significantly and positively associated with tillers/plant (r = 0.56**), grains/spike (r = 0.35**), grain yield/plant (r = 0.56**), seed index (r = 0.66**) and harvest index (r = 0.60**). The chlorophyll content also exhibited fairly good association with yield traits like tillers/plant (r = 0.58**), grains/spike (r = 0.31**), grain yield/plant (r = 0.56**), seed index (r = 0.40**), and harvest index (r = 0.35**). Similar to present findings, Allahverdiyev *et al.* (2015) reported that chlorophyll content was positively and significantly correlated with plant height, spike/m^2 and grain yield. Likewise, plant height, seed weight, spikelets/spike, grains/spike were positively and significantly correlated with most physiological parameters. Therefore, these traits may deem a good criterion for selection.

The correlations between physiological traits were also determined (Table 2) and the results revealed significantly positive correlations between relative water content and chlorophyll content (r = 0.83**) while relative water content and chlorophyll content both were highly but negatively associated with electrolyte leakage (r = -0.91** and r = -0.73**), respectively. These results, by and large, suggested that physiological traits which exhibited high correlations with yield traits in drought condition may be used as selection criteria to select drought tolerant wheat genotypes. Hence, there is a greater scope of using physiological traits along with yield traits in selection for improving yield productivity in water shortage condition.

Conclusion

Ten popular wheat genotypes were evaluated for drought tolerance by imposing water stress at anthesis stage. The results revealed that water stress caused significant decline in all morpho-yield and physiological traits studied. The genotypes Bhitai, Kiran, PBGST-03 and Sarsabz which recorded good performance by giving minimum reductions in majority of the traits under stress were regarded as drought tolerant among ten cultivars that were evaluated. The high positive correlations of physiological traits with almost all yield traits indicated that these physiological traits could serve as reliable criteria for breeding for drought tolerant wheat cultivars.

Table 2. Correlation coefficient (r) between yield and physiological traits of wheat genotypes grown over non-stress and water stress conditions

No.	Correlation between yield traits	Correlation (r)
1	Plant height vs productive tillers/plant	0.39**
2	Plant height vs grains/spike	0.21*
3	Plant height vs grain yield/plant	0.16ns
4	Plant height vs seed index	0.33**
5	Plant height vs harvest index	0.22*
6	Plant height vs grain/yield (kg/ha)	0.26**
7	Productive tillers/plant vs grains/spike	0.29**
8	Productive tillers/plant vs grain/yield plant	0.45**
9	Productive tillers/plant vs seed index	0.15ns
10	Productive tillers/plant vs harvest index	0.24**
11	Productive tillers/plant vs grain/yield (kg/ha)	0.05ns
12	Grains/spike vs grain/yield plant	0.63**
13	Grains/spike vs seed index	0.09ns
14	Grains/spike vs Harvest index	0.10ns
15	Grains/spike vs grain yield kg/ha	0.47**
16	Grain yield/plant vs seed index	0.31**
17	Grain yield/plant vs harvest index	0.37**
18	Grain yield/plant vs grain yield (kg/ha)	0.36**
19	Seed index vs harvest index	0.62**
20	Seed index vs grain yield (kg/ha)	0.42**
21	Harvest index vs grain yield (kg/ha)	0.35**
	Correlation between yield and physiological traits	
22	Plant height vs relative water content	0.49**
23	Plant height vs electrolyte leakage	-0.54**
24	Plant height vs chlorophyll content	0.44**
25	No. of tillers/plant vs relative water content	0.56**
26	No. of tillers/plant vs electrolyte leakage	-0.50**
27	No. of tillers/plant vs chlorophyll content	0.58**
28	Grains/spike vs relative water content	0.35**
29	Grains/spike vs electrolyte leakage	-0.41**
30	Grains/spike vs chlorophyll content	0.31**
31	Grain yield/plant vs relative water content	0.56**
32	Grain yield/plant vs electrolyte leakage	-0.57**
33	Grain yield/plant vs chlorophyll content	0.56**
34	Seed index vs relative water content	0.66**
35	Seed index vs electrolyte leakage	-0.62**
36	Seed index vs chlorophyll content	0.40**
37	Harvest index vs relative water content	0.60**
38	Harvest index vs electrolyte leakage	-0.61**
39	Harvest index vs chlorophyll content	0.35**
	Correlation between physiological traits	
40	Relative water content vs chlorophyll content	0.83**
41	Relative water content vs electrolyte leakage	-0.91**
42	Chlorophyll content vs electrolyte leakage	-0.73**

** = significant at P<0.01 and P<0.05; ns = non significant.

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Pak. j. sci. ind. res. Ser. B: biol. sci. 2016 **59**(3) 126-132

Phenology and Yield of Strawberry as Influenced by Planting Time and Genotypes in a Sub Tropical Region

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(received April 6, 2015; revised June 8, 2016; accepted June 16, 2016)

Abstract. Effects of planting time on phenology of five strawberry genotypes 'Sweet Charlie', 'Festival', 'Camarosa', 'FA 008', and 'BARI strawberry-1' were evaluated at Bangladesh Agricultural Research Institute in two consecutive years 2009-2010 and 2010-2011. 'Sweet Charlie' took the shorter time to begin flowering, followed by 'BARI Strawberry-1' and 'Festival' when planted in 1st October. Genotype 'FA 008' took longer time to flower when planted in 1st December. Days to flowering of all the varieties was found to decrease with the increase in air temperature. Regardless of planting year, the genotype 'FA 005' followed by 'Camarosa' and 'Festival', planted on 1st September, exhibited the longest harvest duration, while 'Sweet Charlie' planted on 1st December exhibited the shortest harvest duration in both years. Genotype 'Festival' planted on October yielded fruit with the greatest fruit weight, followed by 'Sweet Charlie' and 'Camarosa' planted on the same date. Plants of 'FA 008' and 'BARI Strawberry-1' planted in December produced minimum fruit weight. Maximum number of fruits/plant as well as yield/plant obtained from 'Sweet Charlie' planted in October, while BARI Strawberry-1 planted in December yielded the least. With the use of quadratic equation it was estimated that maximum yield was obtained at ambient temperature 18.5 °C then it was decreased with the increase of temperature. Strawberry planted in early October was found to be the most suitable in Bangladesh. Among the studied genotypes, 'Sweet Charlie' was found to be superior in yield and early planting, and 'Camarosa' was suitable for late planting. 'Festival' was found less sensitive to planting date.

Keywords: phenology, strawberry, planting time, sub tropical region

Introduction

Strawberry (*Fragaria* × *ananassa* Duch) is a delicious exotic small fruit crop in Bangladesh. It is photo sensitive as well as thermo sensitive. It has adapted to different environmental conditions and being cultivated across the world (Rice, 1990). The cultivars, which are growing in Bangladesh are short day plants. Environmental conditions prevailing during October to March are suitable for growth and development of strawberry (Ahmad and Uddin, 2012). In Bangladesh there is a cycle of six seasons in a year. Environments of each of every two months are significantly different from the other seasons. The temperature in Bangladesh starts decreasing from the second half of October after which the temperature starts to increase until the end of February. Extreme environmental conditions of March are not suitable for strawberry (Ahmad and Uddin, 2012). Daytime temperatures of about 20-26 °C and night time temperatures of about 12-16 °C, with 8-12 h/day length are optimum for higher yields and quality fruits of strawberry (Darnell, 2003). Planting time thus exposes plants to different growing conditions. High temperature can cause morphological, anatomical, physiological, and biochemical change in plant tissue influencing growth and development of crop (Biscoe and Gallagher, 1978). Early planting encourages vegetative growth. On the other hand late planting exposes plant to high temperature zone of climate cycle which shortens developmental phases and thus reduces yield (Rice, 1990). However, cultivar differences in response to high temperature have not been sufficiently reported. Selection of cultivars is one of the strategies to cope with abiotic climatic stresses like high temperature and change of precipitation pattern (Zheng *et al*., 2009). Therefore, the present study was undertaken to evaluate cultivars in different planting times to prolong strawberry season in sub-tropical regions like Bangladesh.

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Materials and Methods

Experimental site, design, layout and plant production. The study was conducted on the Fruit Research Farm of the Horticulture Research Centre, Bangladesh Agricultural Research Institute, Gazipur, Bangladesh

(Latitude 23° 59' N, Longitude 90° 24' E, altitude 14.33 m) during the winter seasons of 2009-10 and 2010-11. This region is classified as sub-tropical, having hot summers (May to August) and mild winters (December to February). The cumulative rainfall is about 119 mm during August to May with an average relative humidity of 82.9%. The mean maximum and minimum temperatures during the cropping period in which the study was conducted were 26.29 and 15.75 °C, respectively. The daily as well as monthly mean weather data were collected, documented and recorded by Physiology Division, Bangladesh Rice Research Institute, Joydebpur, Gazipur, Bangladesh. Monthly mean weather data during the study period of 2009-10 and 2010-11 are shown in Table 1. The soil on the experimental farm was a clay loam, having a pH of 6.2 (slightly acidic), with low organic carbon (0.95%), phosphorus (9 ppm) and potassium (0.17 meq/100 g soil).

The experimental design was a strip plot with two treatment factors, *viz*. genotype and planting date, replicated three times. Genotypes included 'Sweet Charlie', 'Festival', 'Camarosa', 'FA 008' and 'BARI Strawberry-1' and planting dates were '1st September', '1st October', '1st November' and '1st December'.

Table 1. Monthly mean weather data during the experimental periods for strawberry planted at BARI, Gazipur, Bangladesh (2009-2011)

Year/Month	Temperature (°C)		Relative	Cumulative
	Maximum	Minimum	humidity (%)	rainfall (mm)
2009-2010				
August	27.69	22.01	87.22	463
September	27.93	21.84	86.88	144
October	26.79	19.30	86.31	157
November	25.91	15.32	80.77	15
December	21.67	11.24	79.75	00
January	20.10	9.35	79.25	00
February	23.89	11.51	71.49	15
March	28.37	17.84	66.55	12
April	25.94	21.21	77.46	42
May	28.33	20.89	78.46	234
2010-2011				
August	27.69	22.40	84.93	185
September	26.78	21.91	87.73	64
October	27.05	20.80	85.16	110
November	25.33	16.04	80.88	02
December	21.84	11.29	79.86	53
January	22.64	10.22	81.65	00
February	23.64	13.42	69.33	06
March	28.54	16.87	67.49	15
April	28.42	21.09	76.48	55
May	28.77	18.66	85.31	74

Source: Physiology Division, Bangladesh Rice Research Institute, Joydebpur, Gazipur-1701, Bangladesh.

The experiment was conducted during the winter seasons of 2009-10 and 2010-11. Four planting times were assigned in the main plot and the five strawberry genotypes were assigned in sub plot. The unit plot size was 100 × 1000 cm and the plants were spaced 50 × 40 cm on beds. The main plot (strip) size for planting time was 2250 × 1000 cm. A single bed was used as a single treatment. Beds were raised 30 cm above the ground level with a 50 cm wide drain between beds. Each plot contained a double row accommodating 50 plants. Thirty (±five) days old samples (daughter plant) of different strawberry genotypes were planted on the four planting dates in 2009 and then again on the same dates in 2010.

Selected plants of 'Sweet Charlie', 'Festival', 'Camarosa', 'FA 008' and 'BARI Strawberry-1' were planted in nursery beds for multiplication in July-August, 2009 and 2010. After 15 to 20 days, all plants started to produce runners. Runners were two-node horizontal stems, with clonal 'daughter' plants produced at the distal node. The daughter plants were collected and established in poly bags filled with 50% sterilized sand and 50% cow dung followed by labeling. The established daughter plants were used as clonal propagules for the experiment. The propagules were categorized according to the age, crown size, shape and number of leaves for each treatment. Thirty to thirty five days old Propagules were selected for planting in the experimental field.

During land preparation well decomposed cow dung, urea, TSP, and MP @75 ton, 650 kg, 500 kg and 600 kg were applied per hectare, respectively. All the cow dung, TSP and half of MP fertilizer were applied during bed preparation. Total urea and rest of MP were applied 20 days interval at 5 equal splits from 15 days after planting. After planting, runners were removed every 3 to 4 days. Straw mulch (2-3 cm thick) was applied around the plants, which is a practice that helps conserve soil moisture, decrease weed growth, and promotes fruit quality by preventing berries from laying on the soil surface. Remaining weeds were removed as needed. Overhead irrigation was given whenever necessary to maintain available soil moisture in the field for better plant growth. All other necessary cultural practices and plant protection measures were followed during the entire period of experiment according to BARI (2011).

Strawberries were hand harvested every 2 to 3 days and early in the day, when it was cool. The fruits were harvested at commercial maturity, which is when more than 80% of the fruit surface had turned a red colour. Immediately after harvesting, the strawberries were sorted to eliminate damaged fruit and berries were selected for uniform size and colour for subsequent data collection.

Data were collected from the interior plants within each row to avoid potential border effects. In each sub plot, 40 plants were selected randomly for recording data on different phenological, yield and yield-contributing characters.

Data collected. Days to 50% flowering was estimated as the number of days required from planting to first flower opening among 50% of plants per plot. Days to harvest was determined as the number of days required from first flowering to first harvest. Harvest duration was recorded as the number of days required from first fruit harvest to completion of fruit harvest. Total number of harvested fruits was counted from selected 40 plants of each sub plot throughout the harvesting period and the average value of the total number of fruits/plant was counted. Total weight of harvested fruits from select plants were also determined for each plot. Yield per plant was also determined from selected plants.

Data analysis. Data were analyzed using analysis of variance and regression analysis using MSTAT-C program (Russell and Eisenmith, 1983). The mean comparison was done following the Duncan's Multiple Range Test (DMRT).

Results and Discussion

Days to 50% flowering. In respect of interaction between genotypes and planting time, 'Sweet Charlie' planted on 1st October took the minimum number of days for flowering, which was 68 days in 2009-10 and 64 days in 2010-11. Similar trend of days to flowering was observed in genotypes 'Sweet Charlie' and 'BARI Strawberry-1' (69 and 67 days in 2009-10 and 2010-11, respectively) and 'Festival' (74 and 68 days in 2009-10 and 2010-11, respectively) planted on the same date. Genotype 'FA 008' planted on 1st December took maximum number of days to flowering in each crop year 2009-10 and 2010-11 (Table 2).

Table 2. Interaction effect of planting time and genotypes on phenological traits of strawberry at 2009-10 and 2010-11

Treatment	Days to flowering		Days to harvest		Harvest duration	
	2009-10	2010-11	2009-10	2010-11	2009-10	2010-11
1st Sep. × Sweet Charlie	81^{o}	85^{lmn}	17^{nop}	20^{i-n}	80^{g}	87^{cde}
1st Sep. × Festival	8^{kl}	88^{jk}	20^{i-n}	23^{e-j}	88^{cd}	93^{b}
1st Sep. × Camarosa	87^{kl}	92^{fgh}	23^{e-j}	22^{g-l}	90^{c}	97^{a}
1st Sep. × FA 008	88^{jk}	93^{d-g}	24^{c-h}	26^{b-f}	94^{b}	98^{a}
1st Sep. × BARI Strawberry-1	84^{mm}	83^{no}	20^{i-n}	18^{mno}	85^{ef}	82^{g}
1st Oct. × Sweet Charlie	68^{s}	64^{t}	21^{h-m}	23^{e-j}	80^{g}	72^{i}
1st Oct. × Festival	74^{qr}	68^{s}	23^{e-j}	26^{b-e}	83^{fg}	81^{g}
1st Oct. × Camarosa	75^{q}	72^{r}	23^{e-j}	28^{ab}	85^{def}	83^{fg}
1st Oct. × FA 008	78^{p}	76^{pq}	27^{bc}	31^{a}	85^{def}	88^{cde}
1st Oct. × BARI Strawberry-1	69^{s}	67^{t}	23^{e-j}	19^{k-n}	81^{g}	77^{h}
1st Nov. × Sweet Charlie	78^{p}	83^{no}	24^{c-h}	18^{mno}	50^{lm}	45^{n}
1st Nov. × Festival	84^{mn}	90^{hij}	23^{e-j}	24^{c-h}	53^{k}	49^{m}
1st Nov. × Camarosa	86^{klm}	91^{ghi}	22^{g-k}	27^{bc}	54^{jk}	51^{klm}
1st Nov. × FA 008	88^{jk}	95^{bcd}	25^{b-g}	30^{a}	56^{j}	54^{jk}
1st Nov. × BARI Strawberry-1	78^{p}	83^{no}	19^{k-n}	22^{g-k}	52^{kl}	49^{m}
1st Dec. × Sweet Charlie	88^{jk}	93^{d-g}	15^{opq}	13^{q}	32^{op}	21^{r}
1st Dec. × Festival	90^{hij}	96^{bc}	15^{opq}	17^{nop}	33^{op}	28^{q}
1st Dec. × Camarosa	94^{c-f}	97^{b}	18^{mno}	17^{nop}	34^{o}	30^{pq}
1st Dec. × FA 008	95^{bcd}	99^{a}	17^{nop}	18^{mno}	35^{o}	32^{op}
1st Dec. × BARI Strawberry-1	87^{kl}	92^{fgh}	15^{opq}	14^{pq}	32^{op}	23^{r}
Level of significance	**		**		**	
CV (%)	4.99		6.80		2.04	

Crops of September planting took longer time for flowering, might be due to interaction of temperature and day length. The genotypes cultivating in Bangladesh are photosensitive and flower in short day. In addition to day length, prevailing temperature after planting might encourage vegetative growth (Chercuitte *et al*., 1991). Irrespective of genotype, days to flowering of October planting crops in both the years were optimum. This might be due to the fact that October planting crops got sufficient time to attain proper vegetative growth before perceiving photoperiodic response of short days and resulting in floral evacation (Singh *et al*., 2007). On the other hand, crops of December plantings grew in cool environment and did not get sufficient time to attain proper vegetative growth. Thus, plant age and low ambient temperatures (21.67-21.84 °C and 11.24-11.29 °C, respectively), might have resulted in delaying to flowering. Genotypic responses to the temperature in the growing environment may effect the results (Table 2). This finding corroborated the results of Macit *et al.* (2007).

Relationship between days to flowering and air temperature. Data revealed between days to flowering and temperature shows that there was a linear but negative relationship (Fig. 1A-D). Days to flowering decreased with the increasing of ambient temperature for each planting time. Crops of 5 different planting dates were exposed in different ambient temperatures during their vegetative and reproductive growth stages. It was revealed from the relationship that with the increase of 1 °C temperature, the days to flowering decreased about 2.58, 3.57, 4.26, and 2.71 days for 1st September, 1st October, 1st November and 1st December planting, respectively. The values of coefficient of determination (R^2) for the four planting times indicated that more than 97% of the decrease in days to flowering was due to higher temperature.

Days to harvest. Planting date and genotype interacted significantly in response to days to harvest from flowering (Table 2). 'Sweet Charlie' took the least days to reach harvest when planted in 1st December (13 and 15 days), followed by 'BARI Strawberry-1' when planted in the same date (14 genotype and 15 days) in 2010-11 and 2009-10, respectively. The maximum days to reach harvest maturity occurred in 'FA 008' when planted in 1st October (31 and 27 days in 2010-11 and 2009-10, respectively), followed by 'FA 008' planted in 1st September (30 days in 2010-11 and 25 days in 2009-10 crop seasons). Rahman *et al*. (2014) and Macit *et al.* (2007), also reported late plantings required least number of days to harvest. Fruit set of late planting occurred in lower ambient temperatures which might experience high temperature during maturity. High temperature might induce shortening of developmental phases (Singh *et al*., 2007).

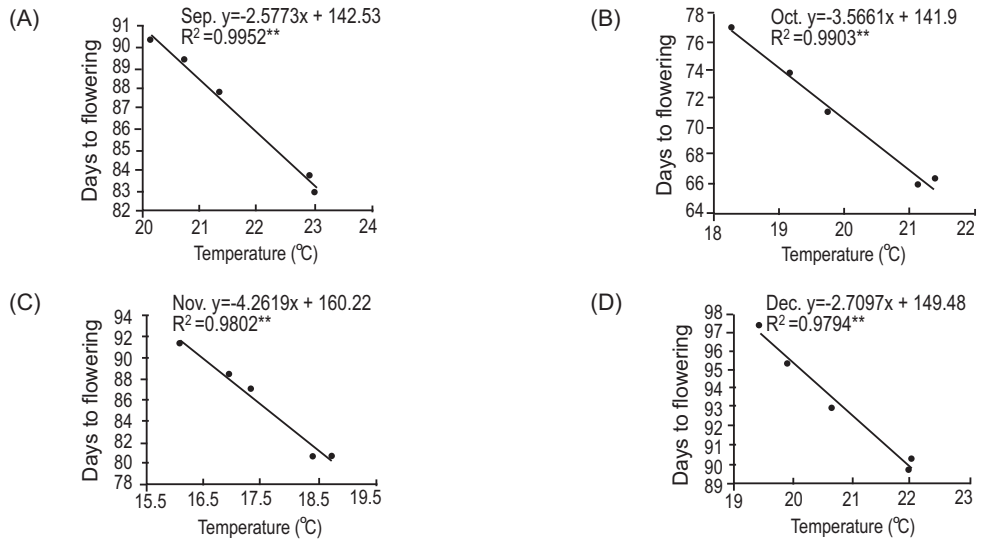

Fig. 1(A-D). Relationship between air temperature and days to flowering for each time of planting when interacted with genotypes (A for 1st September, B for 1st October, C for 1st November and D for 1st December).

Harvest duration. Longer harvest duration was recorded in September planting which was followed by October planting. The shortest harvest duration was recorded in December planting (Table 2). The longest harvest duration was recorded in 'FA 005' (94 and 98 days), followed by 'Camarosa' (90 and 97 days) and 'Festival' (88 and 93 days), planted on 1st September in 2009-10 and 2010-11, respectively. 'Sweet Charlie' planted on 1st December exhibited the shortest harvest duration in both the years (32 days in 2009-10 and 21 days in 2010-11).

Crops of earlier planting dates took the maximum time for fruiting, while late planting crops took less time for fruiting. Ideal temperature and day length of late planting crops might favour early flowering. (Maurer and Umeda, 1999). However, genotypic interaction with temperature were observed in respect of harvest duration (Macit *et al*., 2007).

Fruits per plant. Number of fruits per plant was highest in the crop planted in October and lowest in December planted crop (Table 3).'Sweet Charlie' planted in 1st October produced the maximum fruits/plant (40 fruits/plant in 2009-10 and 38 fruits/plant in 2010-11) followed by 'Festival' (37 and 34 fruits/plant in 2009-10 and 2010-11, respectively), when planted in the same date. In both years, BARI Strawberry-1 when planted on 1st December produced minimum fruits/plant (13 in 2009-10 and 11 in 2010-11). These results are consistent with the findings of Lewis (2003), who reported decline in number of fruits/plant with delayed in planting times. This might be due to the fact that earlier plants were exposed to favourable environment for vegetative growth and subsequently floral signals might be received in November (Table 1). Chercuitte *et al*. (1991) also explained that early planted strawberry plants produced more fruits than late planted plants.

October planting crops attained optimum age and size due to proper vegetative growth and thus achieved the maturity to respond the change of day length for flowering. September planting crops passed a longer vegetative growth period than that of October planting crop and produced a large number of leaves. During reproductive phase, leaves acted as sink and antagonistic for flowering. On the other hand, November and December plantings had less time for vegetative growth and thus

Table 3. Interaction effect of planting time and genotypes on yield and yield contributing characters of strawberry at 2009-10 and 2010-11

Treatment	Fruits per plant		Single fruit wt. (g)		Yield (g/plant)	
	2009-10	2010-11	2009-10	2010-11	2009-10	2010-11
1st Sep. × Sweet Charlie	35^{abc}	33^{a-e}	14.50^{fg}	13.96^{gh}	507.5^{cde}	459.0^{def}
1st Sep. × Festival	32^{a-f}	29^{b-g}	15.15^{de}	14.63^{ef}	484.8^{def}	423.7^{e-h}
1st Sep. × Camarosa	29^{b-g}	26^{d-i}	13.98^{gh}	13.58^{h}	405.4^{e-i}	352.5^{g-j}
1st Sep. × FA 008	22^{g-l}	20^{h-n}	11.00^{kl}	9.90^{mno}	242.0^{k-p}	197.2^{m-r}
1st Sep. × BARI Strawberry-1	24^{f-j}	21^{g-m}	10.50^{lm}	10.18^{mn}	252.0^{k-o}	213.3^{l-q}
1st Oct. × Sweet Charlie	40^{a}	38^{a}	17.50^{b}	16.74^{c}	700.0^{a}	634.4^{ab}
1st Oct. × Festival	37^{ab}	34^{a-d}	18.20^{a}	17.36^{b}	673.4^{ab}	588.7^{bc}
1st Oct. × Camarosa	34^{a-d}	32^{a-f}	17.50^{b}	16.74^{c}	595.0^{bc}	535.1^{cd}
1st Oct. × FA 008	25^{e-j}	23^{g-k}	11.78^{ij}	10.46^{lm}	294.5^{j-m}	239.7^{k-p}
1st Oct. × BARI Strawberry-1	26^{d-i}	24^{f-j}	12.10^{i}	11.46^{jk}	314.6^{i-l}	274.6^{j-n}
1st Nov. × Sweet Charlie	27^{c-i}	24^{f-j}	15.10^{def}	13.80^{h}	407.7^{e-i}	330.5^{h-k}
1st Nov. × Festival	28^{c-h}	26^{d-i}	16.20^{c}	15.36^{d}	453.6^{d-j}	399.0^{f-i}
1st Nov. × Camarosa	26^{d-i}	24^{f-j}	16.50^{c}	14.84^{def}	429.0^{e-h}	354.5^{g-j}
1st Nov. × FA 008	20^{h-n}	17^{j-o}	9.50^{opq}	9.18^{pq}	190.0^{n-r}	161.7^{o-s}
1st Nov. × BARI Strawberry-1	20^{h-n}	19^{i-o}	9.20^{pq}	9.04^{q}	184.0^{n-r}	171.8^{n-s}
1st Dec. × Sweet Charlie	17^{j-o}	15^{k-o}	9.10^{q}	8.24^{s}	154.7^{o-s}	127.5^{qrs}
1st Dec. × Festival	21^{g-m}	17^{j-o}	9.80^{nop}	8.86^{qr}	205.8^{m-q}	151.1^{o-s}
1st Dec. × Camarosa	19^{i-o}	17^{j-o}	9.00^{q}	8.34^{rs}	171.0^{n-s}	139.7^{p-s}
1st Dec. × FA 008	14^{l-o}	12^{no}	6.90^{t}	6.00^{u}	96.60^{rs}	74.27^{s}
1st Dec. × BARI Strawberry-1	13^{mno}	11^{o}	7.10^{t}	6.24^{u}	92.30^{rs}	68.44^{s}
Level of significance	**		**		**	
CV (%)	4.99		6.80		2.04	

delayed in attaining physiological maturity to respond floral induction of short days. The very early planted crops become more vigorous in growth and this subsequently alter C:N ration which hindered flower bud differentiation and thus occurred less production, and lower number of fruits/plant (Chercuitte *et al*., 1991).

Fruit weight. Fruits of 'Festival', 'Sweet Charlie' and 'Camarosa' of October planting crops weighed maximum (18.20 g in 2009-10 and 17.36 g in 2010-11), (17.50 g and 16.74 g in 2009-10 and 2010-11, respectively). FA 008 planted in December yielded fruit with lowest weight (6.90 g) in 2009-10 and (6.00 g) in 2010-11 (Table 3). Shortening of developmental phases induced by high temperature, reduced light perception over the shortened life cycle and perturbation of the processes associated with plant carbon balance have resulted in small fruit size. On the other hand, fruit of September planting crops, had optimum time for physiological development (Rahman *et al*., 2014; Singh *et al*., 2007). Heat stress also reduced the accumulation of sucrose and ascorbic acids and thus affected fruit quality negatively (Hassan *et al*., 2000).

Yield per plant. 'Sweet Charlie' planted in October yielded maximum fruits per plant consistently in both the years. Statistically similar trend of results were followed by 'Festival' (673.4 and 588.7 g per plant in 2009-10 and 2010-11, respectively) and 'Camarosa' (595.0 and 535.1 g per plant in 2009-10 and 2010-11, respectively), planted on the same date, 'Festival' and 'Camarosa' planted in December produced the minimum fruits per plant (Table 3). 'BARI Strawberry-1' had the minimum fruit/plant when planting on 1st December (Table 3). This might be due to sensitivity of the genotype to the planting dates. In a study of planting time and yield of strawberry Chercuitte *et al*. (1991) reported that the cultivar 'Kent' produced the highest yield when planted in early May and lowest when planted in August in Quebec, Canada. Chandler *et al*. (1991) found that 'Dover' strawberry produced its highest fruit yield when planted early, while 'Selva', 'FL-83-37' and 'FL 87-210' were less sensitive to planting time and produced more or less similar yield.

Relationship between yield per plant and air temperature. The result revealed a significant negative relationship between yield per plant and temperature (Fig. 2A-D). Yield per plant was maximum in September and 1st October planting crops when temperature ranges from 20 to 23 °C and 18-21 °C, respectively. It was observed from the regression equations for 1st November and 1st December for every increase of 1 °C temperature, the yield/plant was decreased about 99.8 g and 34.1 g, respectively.

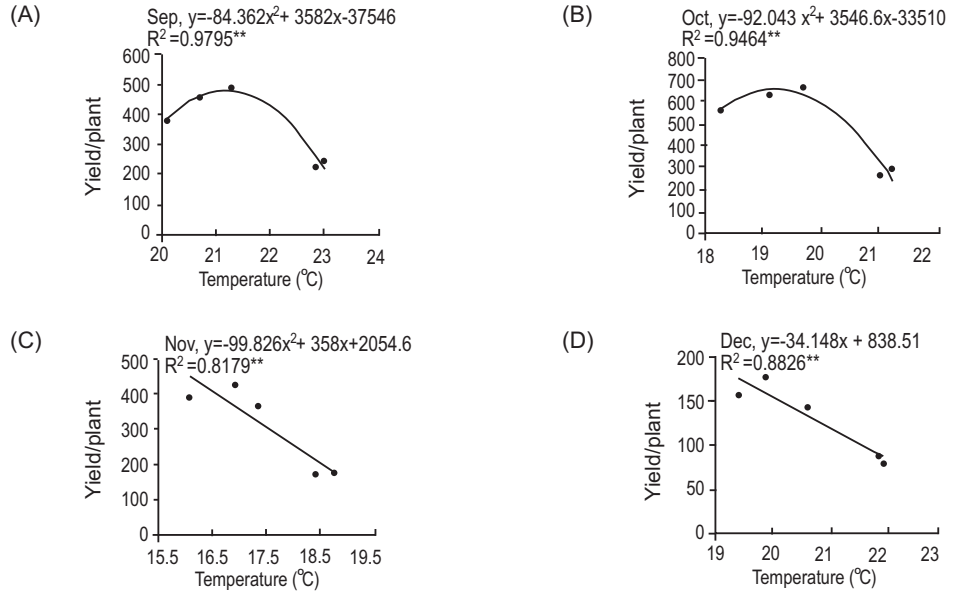

Fig. 2(A-D). Relationship between air temperature and yield per plant for each time of planting when interacted with genotypes (A for 1st September, B for 1st October, C for 1st November and D for 1st December).

Conclusion

Considering phenology, yield contributing characters, and yield irrespective of annual effects, planting on 1st October was found to be the most suitable for strawberry cultivation under subtropical conditions found in Bangladesh. 'Sweet Charlie' was found to be superior in yield and considered the best for early planting, while 'Camarosa' was suitable for later plantings in Bangladesh. 'Festival' was found less sensitive to planting date.

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Pak. j. sci. ind. res. Ser. B: biol. sci. 2016 **59**(3) 133-138

Composition of Soil Seed Bank Over Cholistan Desert Microhabitats at Dingarh Fort Area, Pakistan

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(received May 28, 2015; revised April 12, 2016; accepted April 13, 2016)

Abstract. Soil seed banks were assessed in three soil layers (L1, from 0 to 2 cm, L2, 2 to 4 cm and L3, 4 to 6 cm depth) from five microhabitats i.e., Lee-ward side of sand dune (S1), Wind-ward side of sand dune (S2), Clayey area covered with sand (S3), Interdunal sandy area (S4) and Shifting sand dune of site Dingarh Fort area (S5) in Cholistan desert of Pakistan to analyse differences of soil seed bank among these habitats. Ten soil samples were collected from each microhabitat and from each layer i.e., 0-2 cm depth (L1), 2-4 cm depth (L2) and 4-6 cm depth (L3) by using 15×15×6 cm metallic sampler. Consistent differences in seed composition were observed among these microhabitats. Seedling emergence approach was used to assess the soil seed bank of Cholistan desert. Canonical correspondence analysis (CCA) was used for the soil seed bank and the plant species analysis. The microhabitats S3 (Clayey area covered with sand) and S4 (Interdunal sandy area) contributed prominently to the total variance in the species and had maximum density of seed bank and soil layer L1 contained maximum number of seeds.

Keywords: Cholistan desert, seed bank, microhabitat, canonical correspondence analysis

Introduction

Cholistan is a hot and sandy desert in South of Punjab province of Pakistan (Arshad *et al*., 2002). The mean annual rainfall ranges from < 100 mm in the West and up to 200 mm in the East. Rain usually occurs during monsoon (July-September) and spring (January- March). Average minimum and maximum temperature ranges between 20 °C and 40 °C, with highest soaring up to 50 °C (Arshad *et al*., 2002; Mughal, 1997). As annual rainfall is highly variable both on temporal and spatial scales, aridity is the most striking feature of this desert with wet and dry years occurring in clusters. Information of soil seed banks and their relationship to the standing vegetation of this area is a fundamental part of our scientific knowledge and understandings about the ecological and physiological processes through which all plants in general and desert plants in particular have become adapted to their variable and harsh environment. Although the literature related to seed banks is increasing and expanding (Mandák *et al*., 2012; Zuo *et al*., 2012; Schütz *et al*., 2008; Wolters and Bakker, 2002; Gul and Khan, 2001; Khan, 1993; Benoit *et al.,* 1989; Egley, 1986) however, the present literature has no evidence about the seed bank of Cholistan desert of Pakistan. A major generalization of desert ecology is that the number of seeds per unit area in the soil seed bank remarkably varies among microhabitats, so far as in any desert different microhabitats can be selected for analysing the seed bank. Assessing differences in seed composition of distinct microhabitats can clarify the relationship between patterns and processes of soil seed banks. Knowledge of the particular location of different seed types may explain the spatial pattern of plant recruitment at the microhabitat scale (Marone *et al.,* 2000). The detection of differences in composition of soil seed banks, but not of gross differences in total seed numbers, can help to identify the main ecological mechanisms which govern seed fluxes in arid and semiarid ecosystems of deserts like Choilstan. From 80 to 90% of soil seeds are present in the upper 2 cm of soil (Al-Yameniand Farraj, 1995; Reichman, 1975; Childs and Goodall, 1973) and of those, most are in the litter or top few centimeters of soil (Bastida *et al.,* 2010; Young and Evans, 1975). The information on how spatial scale influences the spatial heterogeneity of soil seed banks in a grassland under grazing disturbance is still lacking (Zuo *et al*., 2012). Same is the case with the information about the soil seed bank of Cholistan desert, although the soil seed bank is very important for conservation

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of species (Bernhardt and Elisabeth, 1989). So, the present study was under taken to observe seed bank of Cholistan desert and results of this study can provide useful information for the conservation of biodiversity of Cholistan desert.

Materials and Methods

Seed bank samplings. The present ecological piece of work was conducted at Baghdad-ul-Jadeed campus, The Islamia University of Bahawalpur, Pakistan to find out the viable seed bank for the soil samples from a selected site in Dingarh Fort area in Cholistan desert. Soil samples for seed bank were collected from three layers. They were 0-2 cm (L1), 2-4 cm (L2) and 4-6 cm (L3) of five microhabitats including (i) Lee-ward side of sand dune (S1), (ii) Wind-ward side of sand dune (S2), (iii) Clayey area covered with sand (S3), (iv) Interdunal sandy area (S4) and (v) shifting sand dune (S5). The field area of University campus also has sand dunes like that of Cholistan desert where temperature becomes so high in summer (40-50°C) as in desert and rainfall is mostly below 200 mm, which was ideal place for this experiment.

These samples were placed in the plastic bags and were brought to the laboratory of Cholistan Institute of Desert Studies (CIDS), The Islamia University of Bahawalpur. Samples were spread in 15×15 cm plastic trays of 4 cm depth, which were made by plastic sheets molded by hand and amended to experimental trays. The required amount of water was provided regularly for seed germination to estimate the seeds viability. In the present study, the seedling emergence approach was adopted to assess the soil seed bank of Cholistan desert. Two estimation methods of soil seed bank i.e., (i) the physical extraction of seeds from the soil and (ii) emergence of seedlings, were used to estimate seed bank composition (Brown, 1992). Seedlings were identified physically and visually which were later on counted on simple numbering basis. Data was statistically analysed by using the Canoco statistical analysis programme (Braak and Šmilauer, 2002).

Results and Discussion

The interpretations of multivariate results using Canonical correspondence analysis (CCA) are straight forward and largely graphical (Palmer, 1993; Braak, 1986). The ordination diagram for Canonical correspondence analysis using the programme Canoco (Braak and Šmilauer, 2002; Braak and Prentice, 1988) was carried out on the quantitative seed bank data.

The seed bank data about Dingarh Fort area indicates that the seed of most of the species were present in the centre for all the four axis with the exception of *Acacia nilotica* present on the extreme left of the diagram nearer

Table 1. Weighed (weight = sample total) correlation matrix of ordination axis, seed bank and environmental variables Dingarh Fort area (DGA)

SPEC AX1	1.0000	-	-	-	-	-	-	-
SPEC AX2	0.0591	1.0000	-	-	-	-	-	-
SPEC AX3	-0.1536	-0.0128	1.0000	-	-	-	-	-
SPEC AX4	-0.0994	-0.0698	0.0628	1.0000	-	-	-	-
ENVI AX1	0.9000	0.0000	0.0000	0.0000	1.0000	-	-	-
ENVI AX2	0.0000	0.9431	0.0000	0.0000	0.0000	1.0000	-	-
ENVI AX3	0.0000	0.0000	0.8386	0.0000	0.0000	0.0000	1.0000	-
ENVI AX4	0.0000	0.0000	0.0000	0.9129	0.0000	0.0000	0.0000	1.0000
S1	0.1549	-0.1418	-0.3947	0.6840	0.1721	-0.1504	-0.4707	0.7493
S2	0.7915	0.1139	-0.0938	-0.2603	0.8795	0.1208	-0.1119	-0.2851
S3	-0.4668	0.5694	0.2098	-0.2406	-0.5187	0.6038	0.2502	-0.2636
S4	-0.1199	-0.4586	-0.3065	-0.3462	-0.1332	-0.4863	-0.3655	-0.3793
S5	0.2364	-0.2545	0.7046	0.1465	0.2627	-0.2698	0.8403	0.1605
L1	-0.1159	-0.6378	0.0042	-0.3060	-0.1288	-0.6763	0.0050	-0.3352
L2	0.0170	0.2033	0.0518	0.4504	0.0189	0.2156	0.0617	0.4934
L3	0.1546	0.7093	-0.0731	-0.1139	0.1718	0.7521	-0.0871	-0.1248
	SPEC AX1	SPEC AX2	SPEC AX3	SPEC AX4	ENVI AX1	ENVI AX2	ENVI AX3	E N V I AX4

Table 2. Weighed correlation matrix of environmental variables (microhabitats and soil depth) of seed bank, Dingarh Fort area, (DGA)

S1	1.0000	-	-	-	-	-	-	-
S2	-0.1001	1.0000	-	-	-	-	-	-
S3	-0.4790	-0.2017	1.0000	-	-	-	-	-
S4	-0.2413	-0.1016	-0.4859	1.0000	-	-	-	-
S5	-0.1436	-0.0605	-0.2892	-0.1457	1.0000	-	-	-
L1	0.0554	-0.1106	-0.0810	0.0613	0.0596	1.0000	-	-
L2	-0.0277	0.0696	0.0139	0.0068	-0.0459	-0.7590	1.0000	-
L3	-0.0486	0.0788	0.1055	-0.1020	-0.0316	-0.5453	-0.1318	1.0000
	S1	S2	S3	S4	S5	L1	L2	L3

Table 3. Eigenvalues and cumulative percentage variances of seed bank, Dingarh Fort area

Axes	1	2	3	4	Total inertia	Axes	1	2
S1	1.0000	-	-	-	-	-	-	-
S2	-0.1001	1.0000	-	-	-	-	-	-
S3	-0.4790	-0.2017	1.0000	-	-	-	-	-
S4	-0.2413	-0.1016	-0.4859	1.0000	-	-	-	-
S5	-0.1436	-0.0605	-0.2892	-0.1457	1.0000	-	-	-
L1	0.0554	-0.1106	-0.0810	0.0613	0.0596	1.0000	-	-
L2	-0.0277	0.0696	0.0139	0.0068	-0.0459	-0.7590	1.0000	-
L3	-0.0486	0.0788	0.1055	-0.1020	-0.0316	-0.5453	-0.1318	1.0000
	S1	S2	S3	S4	S5	L1	L2	L3

to axis1 i.e., the environmental variable (microhabitat) S2 (Wind-ward side of sand dune). Spearmans correlation coefficients of this data presented in Table 1-2, show relationship among ordination axis, seed bank of species and the environmental variables. The Eigenvalues for species-environment correlations were 0.90, 0.94, 0.84, 0.91 for axis 1 to axis 4, respectively (Table 3).

Here at this site the total number of species in seed bank were 19, out of which12 were annuals with 7 perennials. Among the 12 annuals, 3 were grasses while there were 3 perennial grasses among the 7 perennials. The total inertia (total variance in the species data) was recorded as 0.98 (Table 3). Out of the total five microhabitats involved in this analysis, only two, S3 (Clayey area covered with sand) and S4 (Interdunal sandy area) contributed prominently to the total variance in the species data while out of the three layers of soil, only L1 (0-2 cm depth) contributed the maximum to total species variance. The strong differences in the soil seed bank composition among these microhabitats, and three surface layers, studied at this site depicted by weighed means (0.49), standard deviation (0.50), and Inflation factor (3.66). The maximum values for S3 (Clayey area covered with sand) and these three characters were also highest for L1 (0-2 cm depth) i.e., 0.76, 0.43 and 2.42, respectively (Table 4).

The species *Cenchrus biflorus*, *Cenchrus ciliaris*, *Aristida funiculata*, *Tribulus longipetalus*, *Mollugo cerviana*, *Indigofera hocstetteri*, *Euphorbia prostrata*, *Gisekia pharnaceoides*, *Dipterygium gluacum, Capparis decidua*, *Calligonum polygonoides* and *Ochthochloa compressa* were found much associated to S3 and the soil layer L1, as they had maximum number of seeds. The weakest association was exhibited by environmental variable S5 and soil layer L3 having the minimum species in soil seed bank with lowest number of seeds as shown in the ordination diagram (Fig.1).

Seed bank. Indigenous soil seed banks play a very important role in facilitating the natural vegetation particularly in the deserts where the environmental conditions are too harsh and recovery of vegetation after a long dry spell mainly depends on the soil seed banks. Desert ecosystems rely heavily on the remaining soil seed bank as a reservoir of plant propagules. The

Table 4. (Weighed) mean, standard deviation and inflation factor, Dingarh Fort area (DGA)

Name	Mean (weighed)	Stand. dev.	Inflation factor
SPEC AX1	0.0000	1.1112	-
SPEC AX2	0.0000	1.0603	-
SPEC AX3	0.0000	1.1925	-
SPEC AX4	0.0000	1.0954	-
ENVI AX1	0.0000	1.0000	-
ENVI AX2	0.0000	1.0000	-
ENVI AX3	0.0000	1.0000	-
ENVI AX4	0.0000	1.0000	-
S1	0.1921	0.3940	2.7552
S2	0.0404	0.1970	1.4684
S3	0.4910	0.4999	3.6639
S4	0.1966	0.3974	2.7890
S5	0.0798	0.2709	0.0000
L1	0.7584	0.4280	2. 4211
L2	0.1551	0.3620	2.3808
L3	0.0865	0.2811	0.0000

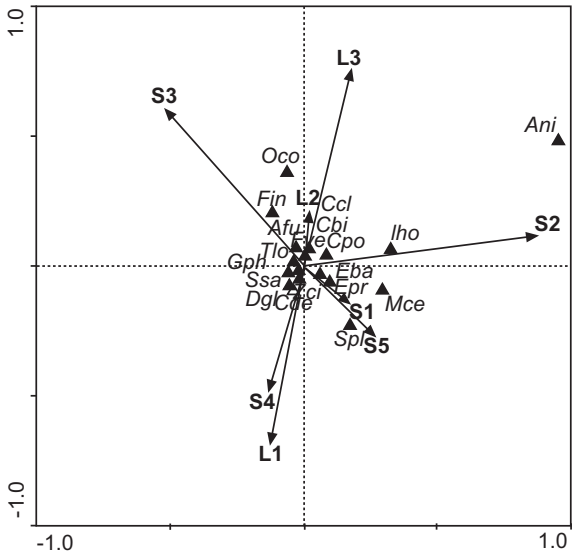

Fig. 1. Canonical correspondence ordination of seed bank at site Dingarh Fort area (CCA seed bank data) showing the relationships between species (triangles) and environmental variables (arrows) *Eragrostis barreliri*= Eba,*Ochthochloa compressa*=Oco; *Cenchrus biflorus*=Cbi; *Cenchrus ciliaris* =Cci; *Aristida funiculata*=Afu; *Tribulus longipetalus*=Tlo; *Sasuvium sasuvioides*= Ssa; *Mollugo cerviana* =Mce; *Indigofera hocstetteri*= Iho; *Gisekia pharnaceoides*= Gph; *Euphorbia prostrata*=Epr, *Foeniculum velgarus*=Fve; *Dipterygium gluacum*=Dgl; *Capparis decidua*=Cde;*Calligonum polygonoides* =Cpo; *Acacia nilotica*=Ani; *Fagonia cretica*=Fin, *Stipagrostis plumosa*= Spl. The environmental variables (vectors) (microhabitats)=S1; (Lee-ward side of sand dune) S2 (Wind-word side of sand dune), S3 (Clayey area covered with sand)=S4 (Interdunal sandy area)=S5 (Shifting sand dune) and three soil layers=L1 (0-2 cm depth); L2=(2 to 4 cm); L3= (4-6 cm).

goal of this study was to determine the size of the soil seed bank and distribution of the seeds at different depths in five different microhabitats of site Dingarh Fort area in Cholistan desert. There are two main ways to estimate the soil seed bank. The physical extraction of seeds from the soil and emergence of seedlings from soil are used to estimate seed bank composition (Brown, 1992). In present study the seedling emergence approach was used to assess the soil seed bank of Cholistan desert.

Canonical correspondence ordination of seed bank at Dingarh Fort area (CCA seed bank data) showed that ephemerals (annuals) have large amount of seeds in the soil seed bank both as number of seeds per species and total number of species. These results are in conformity with the findings of Caballero *et al*. (2003) and Bertiller (1998), who described high number of species (68), in the seed bank being most of them annual gypsophytes and perennials play a secondary role here. Annual species were dominant, both in number and density. These results also match seed banks in arid ecosystems studied by Gutierrez *et al*. (2000), Lyaruu and Backeus (1999); Marone *et al*. (1998); Moro *et al.* (1997) and Coffen and Lauenroth (1989).

In this study, the soil seed bank of 19 species was observed in Dingarh Fort area, which comprises of mainly annuals and perennials and only one tree species (Fig. 2) (Schutz *et al*., 2008; Coffin and Lauenroth, 1989). Among the five microhabitats of Dingarh Fort area the microhabitats S3 (Clayey area covered with sand) and S4 (Interdunal sandy area) contributed prominently to the total variance in the species and have maximum seed bank and soil layer L1 (0-2 cm depth) contained the maximum number of seeds as observed by Al-Yemeni and Ferraj (1995) that maximum emergence of seedlings form upper most (0-2 cm) layer of the soil. Also many other observations on the vertical distribution of the seed bank confirmed literature reports that the majority of seeds are located in the top layer of the soil (Gul and Khan, 2001; Marone *et al*., 1998; Connor and Pickett, 1992).

Fig. 2. Plastic sheets molded and amended to experimental trays, showing the germination of seedlings of *Acacia nilotica* (tree) *and Cenchrus ciliaris* (grass). Picture shows the successful vegetative survival of both types of seedlings, three weeks after their germination.

The weakest association was exhibited by microhabitat S5 (Shifting sand dune) and soil layer L3 (4-6 cm) having the minimum species in soil seed bank with lowest number of seeds. Ma *et al*. (2006) reported that microhabitat and stand age of sand have effects on soil seed viability and seedling development. They found that three species, namely; *Artemisia ordosica*, *Caragana korshinskii* and *Hedysarum scoparium* exhibited significantly different number of viable seeds in soils from differently aged stands. So according to these findings shifting sand dunes are formed by the moving sand which has very short age so it contained very low soil seed bank, as depicted by the results of this study.

Conclusion

The number of species and emerging seedlings in the soil seed banks were higher in the soil of microhabitat Clayey area covered with sand followed by the microhabitat Interdunal sandy area and Shifting sand dune microhabitat had very low soil seed bank. Among the three soil layers L1(0-2 cm) had maximum soil seed bank as compared to the deeper layers. So from the above results, it is concluded that different microhabitats had variable soil seed bank and the maximum seed density could occur and prevail in the surface layer of the soil up to 2 cm depth.

Acknowledgement

We acknowledge Major Tahir for providing luggage support in sample collections and Prof. Dr. Muhammad Mukhtar, Vice Chancellor, The Islamia University of Bahawalpur, for his fruitful facilitation by providing computer and canoco programme, during the course of investigations.

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Pak. j. sci. ind. res. Ser. B: biol. sci. 2016 **59**(3) 139-145

Biochemical Characterisation and Dietary Fibre Analysis of Sugar Beet Supplemented Cookies

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(received February 1, 2016; revised May 12, 2016; accepted May 19, 2016)

Abstract. This study was planned to utilize sugar beet powder as a rich source of dietary fibre in cookies. Purposely, five treatments namely T_1, T_2, T_3, T_4 and T_5 with 4%, 8%, 12%, 16% and 20% sugar beet powder addition in wheat flour were chosen to estimate fibre, antioxidant profiling and engineering properties of cookies. Results showed an increased content of all above mentioned parameters. With the increment in sugar beet powder addition in treatments, dietary fibre analysis have shown that total dietary fibre (TDF), insoluble dietary fibre (IDF) and soluble dietary fibre (SDF) have depicted increasing trend with maximum for T_5 for all dietary fibre types. Significant results were obtained for *in vitro* antioxidant studies including total phenolic content (TPC) and DPPH that showed increasing trend with T_1 0.6 mg GAE/g and maximum values for T_5 with 2.0 mg GAE/g for TPC and for DPPH with T_5 being maximum value of 1.7% and minimum for T_1 with 1.3%. T_5 treatment with 20% sugar beet gave best physicochemical results but disturbed sensory properties while T_3 with 12% sugar beet powder showed good physicochemical and sensory characteristics. Therefore, T_3 with 12% level is considered as the best source of dietary fibre in bakery products and can be considered as the prospective choice to address metabolic syndromes.

Keywords: sugar beet, dietary fibre, biochemical characterisation, cookies, sensory analysis

Introduction

Changing dietary patterns have led to preparation of certain health endorsing foods. Amongst, dietary fibre holds paramount importance in addressing various lifestyle related disorders.

In this regard people put their health at stake and adopt such dietary patterns that are health deteriorating leading to various ailments. In this regard they use junk foods that are deficient in dietary fibres. Therefore, to fulfill this need, sugar beet can be used as a good dietary fibre provider.

Sugar beet (*Beta vulgaris*) a good source of energy, nutrition and dietary fibre belongs to the family Chenopodiaceae (Ahmad *et al*., 2012). It has many varieties of different shapes and colours. Sugar beet has a characteristic barn, silage like, musty or strong earthy odour because of two main compounds namely: geosmin (tarns-l,10-dimemyl-trans-(9)-decalol) and 2-methoxy-3-sec-butylpyrazine (Lu *et al*., 2003). It is a potential source of phenolic and potent antioxidant compound with significant amount of various vital phenolic acids (Vulic *et al*., 2012) with chlorogenic, gallic, gentisic, ferulic and coumeric acids (Belal, 2007; Sakac *et al*., 2004; Brand-Williams *et al*., 1995). Ferulic acid, a potent antioxidant is much higher in sugar beet as compared to wheat flour and wheat bran.

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It is a good source of minerals and vitamins especially; vitamin A, C, folate, potassium, sodium, magnesium, calcium, iron, copper and zinc (Skrbic *et al*., 2010). It contains betaine along with folic acid, vitamin B_{12} and vitamin B_6 which helps to reduce higher levels of homocysteine (Schnyder *et al*., 2002). Sugar beet is also high in bio-flavonoids and has half sweetness than sucrose therefore more suitable for hyperglycemic patients (Vulic *et al*., 2012).

Almost 70% total dietary fibre of sugar beet composed of 1/3rd soluble fibre and 2/3rd insoluble fibre (Filipovic *et al*., 2007). Being a good source of dietary fibre it reduces the risk of cancers, obesity, diabetes, gallstone formation and heart diseases; prevents and treats constipation and cures diverticulitis (Ahmad *et al*., 2012). Besides providing health benefits, it also performs certain functional properties in food products i.e., it can act as antioxidant, anticaking, binding, bulking, dispersing, thickening, stabilizing and texturizing agent in various food products like patties, sauces, sausages, snacks, fruit fillings, yogurt, beverages and bakery (Ralet *et al*., 2009; Thibault *et al*., 2001).

Due to its various health benefits there is an increasing demand of dietary fibre in food products. Recommendations for daily dietary fibre (DF) intake vary from country to country with a range 18-38 g/day, of which 38 g/day is for men and 26 g/day is recommended for women (Rodriguez *et al*., 2006). Based on 2010 con-sumption data, sugar beet fibre consumption by humans was about 97522.35 kg of which about 29545 kg approx. was consumed in the form of baked goods such as bread, cakes and cookies while, 59090 kg was consumed in health products in the form of fibre tablets (Galisteo *et al*., 2008).

Fibre from sugar beet can be better utilised in bakery products by making its composite with wheat flour. In bakery products cookies have been recommended as a better utilisation of composite flour than bread because of their ready to eat form, excellent eating quality, and greater consumption and extended shelf life (Okpala and Chinyelu, 2011; Piga *et al*., 2005). Since cookies are usually prepared from wheat flour with about 72% extraction rate i.e., without bran and germ which causes low dietary fibre content (<2.5%), low protein content (7 to 10%) lacking certain essential amino acids (lysine, tryptophan and threonine), low vitamin and mineral content of cookies (McWatters *et al*., 2003).

The main objective to produce composite flour is to acquire a product which is better than its individual components, improved performances and economy. Composite flour knowledge has been used as a tool for extending short supplies of wheat and corn in the formulation of baked products (Okpala and Chinyelu, 2011; Piga *et al*., 2005).

In the present study, sugar beet powder is being used as a fibre source in cookies formulation. For this purpose its powder was replaced by wheat flour at various substitution levels to produce composite flour. The research of Thibault *et al*. (2001) indicated that sugar beet fibre with its odourless and colourless properties has potential to be used as a dietary fibre source in cookies but exceeding its level from 16% disturbed sensory properties of cookies. Various studies indicated that more than 20% addition of sugar beet alters the rheological properties of bakery products. This composite flour is being used in cookies preparation with the objective of better nutrition and a fibre enriched end product with excellent overall acceptability.

Materials and Methods

Procurement of raw material. Commercially available varieties of sugar beet were purchased from Ayub Agricultural Research Institute (AARI), Faisalabad. Chemicals were purchased from Sigma Aldrich and local market.

Preparation of sugar beet powder. Sugar beets were washed to remove adhering contaminants followed by peeling and cossette preparation. The cossettes were dried by dehydrator as described by Filipovic *et al*. (2007) at 30-35 °C for 24 h and coarsely powdered by grinder, sieved and then stored in polythene bags.

Chemical analyses. The commercial white wheat flour and sugar beet powder were tested for their proximate analyses according to AACC (2000) i.e., moisture content with method No. 44-15A, crude protein with method No. 46-10, crude fat 30-10, crude fibre method No. 32-10 and ash content with method No. 08-01.

Dietary fibre analyses. The sugar beet powder and the cookies were analysed for total dietary fibre, soluble dietary fibre and insoluble dietary fibre according to AACC (2000) as mentioned and described below:

Total dietary fibre (TDF). The sugar beet powder and the cookies were analysed for total dietary fibre according to AACC (2000) method No. 32-05. The sample was dispersed in a buffer solution and incubated with heat-stable α-amylase at 95-100 °C for 40 min. After cooling the samples up to 60 °C, these contents were incubated at 60 °C for 30 min with addition of 100 µL protease solutions. Finally contents were incubated with amyloglucosidase enzyme at 60 °C for 30 min. Total dietary fibre was precipitated with the addition of ethyl alcohol in 1:4 ratio. The contents were filtered and washed with ethyl alcohol and acetone. A blank sample was run throughout entire method with samples to determine any contribution from reagents to residue. The TDF was calculated by the following formula:

$$(\%)\ \text{TDF} = \frac{\text{Residue wt - protein - ash - blank}}{\text{Sample weight}} \times 100$$

Soluble dietary fibre (SDF). The samples were investigated for soluble dietary fibre by following the method as given in AACC (2000) method No. 32-07, by utilising Megazyme assay kit (Megazyme, Ireland). The samples were dispersed in buffer solution and incubated with heat stable α–amylase at 95-100 °C for 35 min. After

cooling, samples were again incubated with addition of 100 µL protease solution at a temperature of 60 °C for 30 min. Finally, the residue was incubated with amyloglucosidase at a temperature of 60 °C for 30 min. After filtration, residue was washed and rinsed with 10 mL distilled water. The filtrate was weighed and soluble dietary fibre (SDF) was precipitated with four volumes of ethyl-alcohol. The contents were filtered, dried and analysed for protein and ash content. A blank sample was also run through entire protocol along with samples to observe any contribution from reagents to residues. The soluble dietary fibre was calculated with the following expression:

$$(\%)\ \text{SDF} = \frac{\text{Residue wt - protein - ash - blank}}{\text{Sample weight}} \times 100$$

Insoluble dietary fibre (IDF). Insoluble dietary fibre (IDF) in different samples was estimated by using the method as mentioned in AACC (2000) method No. 32-20. The samples were dispersed in a buffer solution and incubated with heat stable α-amylase at 95-100 °C for 35 min. After cooling, contents were again incubated with addition of 100 µL protease enzyme at 60 °C for 30 min and then the contents were incubated with amyloglucosidase enzyme at 60 °C for 30 min. After filtration, remaining material was washed and rinsed with 10 mL water. The resultant residue was weighed and insoluble dietary fibre was precipitated with four volume of ethyl-alcohol. The contents were filtered, dried and corrected for protein and ash content. A blank was also run through same method to measure any contribution from reagents to residue. The IDF was calculated by the following expression:

$$(\%)\ \text{IDF} = \frac{\text{Residue wt - protein - ash - blank}}{\text{Sample weight}} \times 100$$

Antioxidant potential of sugar beet. Antioxidant potential of sugar beet was determined by following their respective methods. The free phenolic acids in sugar beet powder were extracted using the method described by Sakac *et al*. (2010). Sugar beet powder 10 g was mixed with 100 mL of 96% ethanol. This mixture was shaken at room temperature for 1 h using orbital shaker. Extract was filtered through Whatmann #1 filter paper and then dried. Dried extract was again dissolved in 96% ethanol up to 10 mL final volume using distilled water. Extracted contents were used for the determination of total phenolic content and radical scavenging activity using DPPH method.

Determination of total phenolic content (TPC). The total phenolic compounds in sugar beet powder and cookies were estimated by some modifications in the Folin-Ciocalteu method (FCM) described by Sakac *et al*. (2010) and Ainsworth and Gillespie (2007). From a known concentration of the sample solution, 125 µL sample was taken in test tube. Then 500 µL distilled water was added in it. After that 125 µL of Folin-Ciocalteu reagent was added in it and given a standing time of almost 6 min. Then 1.25 mL of 7% sodium carbonate was added in it. Final volume was made 3 mL by adding 1 mL of distilled water and given 90 min for completion of reaction.

Absorbance of the samples was measured in triplicate at 750 nm using a UV-Vis spectrophotometer (IRMECO Germany). Gallic acid was run as a standard along with the samples and its absorbance was also taken at 750 nm. TPC was calculated by the following formula:

$$C = c \times V/m$$

where:

C = total contents of phenolic compounds (mg/GAE/g)
c = concentration of gallic acid (mg/mL)
V = the volume of the extract
m = weight of extract (g)

Radical scavenging activity by using DPPH method. The antioxidant activity of ethanolic extract of sugar beet powder and cookies was determined based on the radical scavenging ability in reacting with a stable DPPH free radical (Mohdaly *et al*., 2010; Sakac *et al*., 2010). In this method, 4 mg of DPPH was dissolved in 100 mL methanol and 2 mL of this solution was added to 50 µL ethanolic extract. The mixture was shaken vigorously and allowed to stand at room temperature in the dark for 60 min. Then the absorbance was measured at 517 nm against blank. The radical scavenging percentage was calculated using the following equation:

$$\text{Reduction of absorbance (\%)} = [(AB - AA) / AB] \times 100$$

where:

AB = absorbance of blank sample (t = 0 min)
AA = absorbance of tested extract solution (t = 15 min)

Cookies preparation. Cookies were prepared according to the treatment plan shown in Table 1 by following the method no. 10-54 as mentioned in AACC (2000) from

wheat flour–sugar beet powder composite flour using different percentages as described below:

T_0 = 100% wheat flour; T_1 = 96% wheat flour + 4% sugar beet powder; T_2 = 92% wheat flour + 8% sugar beet powder; T_3 = 88% wheat flour + 12% sugar beet powder; T_4 = 84% wheat flour + 16% sugar beet powder; T_5 = 80% wheat flour + 20% sugar beet powder

Sensory evaluation of cookies. Cookies were analysed according to the procedure described by Lawless and Heymann (2010).

Statistical analysis. The collected data was statistically analysed according to the procedure described by Montogomery *et al*. (2008). The design applied on the data obtained is completely randomized design (CRD).

Table 1. Treatments used for the product development

Treatments	Wheat flour (%)	Sugar beet powder (%)
T_0	100	-
T_1	96	04
T_2	92	08
T_3	88	12
T_4	84	16
T_5	80	20

Results and Discussion

The present project was designed to investigate various physicochemical attributes of sugar beet powder. The main intention of present project was to optimize sugar beet powder quantity as a potential fibre source in cookies production and evaluation of sugar beet fibre cookies for sensory, physical and quality parameters.

Chemical composition of wheat flour. The proximate composition of wheat flour and of sugar beet powder is shown in Table 2. The results pertaining proximate composition of wheat flour revealed that the white flour contained 11% moisture, 1.4% total ash, 10% crude protein, 2.5% crude fat, 0.5% crude fibre and 73.2% NFE content. The results obtained in the present study for the analyses of wheat flour are in close agreement with Ahmad *et al*. (2009), who analysed different wheat varieties and observed 12.5 to 14.6% moisture, 8.23 to 12.71% protein, 1.17 to 1.59% fat, 0.42 to 0.76% crude fibre and 0.42 to 0.66% ash content. Kendler (2006) also analysed wheat varieties for their proximate composition and found that values ranged from 12.49 to 13.27% for moisture, 10.84 to 11.98 % for protein, 0.68 to 0.96 % for crude fibre and 0.40 to 0.58 % for ash contents.

The proximate analysis of sugar beet powder revealed that it contained 5.1% moisture, 5.0% ash, 6.1% crude protein, 0.8% crude fat, 9.0% crude fibre and 74% NFE content. The results obtained in the present study for the proximate analysis of sugar beet powder are in accordance to the findings of McWatters *et al*. (2003) who observed 6.8% crude protein, 0.6% crude fat and 5.5% ash content, with previous study of Westenhoefer (2001) who analysed 4.6% moisture content in sugar beet and also in close agreement with previous study of Belal (2007) who found 12.73 and 18.7% crude fibre in different sugar beet varieties, respectively.

Total dietary fibre, soluble dietary fibre and insoluble dietary fibre content in sugar beet powder was 31%, 10% and 21%, respectively as shown in Table 2. These findings were in consistence with the outcomes of other researchers (Sakac *et al*., 2010; Filipovic *et al*., 2007). Total phenolic content of sugar beet powder was expressed in terms of gallic acid equivalent (GAE) per gram of sugar beet powder from which the extract was obtained and was found to be 1.18 mg GAE/g. The values of total phenolic content in this study are in accordance with the values as observed by Mohdaly *et al*. (2010). DPPH radical scavenging activity of sugar beet powder was expressed in dry weight percentage

Table 2. Chemical composition of commercial white flour and sugar beet powder

Characteristics	Commercial white flour	Sugar beet powder
Moisture (%)	11.0 ± 0.1	5.10 ± 0.1
Crude protein (%)	10.0 ± 0.2	6.10 ± 0.4
Ash (%)	1.40 ± 0.1	5.00 ± 0.6
Crude fibre (%)	0.50 ± 0.1	9.00 ± 0.1
Crude fat (%)	2.50 ± 0.2	0.80 ± 0.1
NFE (%)	73.2 ± 0.3	74.0 ± 0.6
TPC (mgGAE/g)	-	1.18 ± 0.8
DPPH (%)	-	79.1 ± 0.2
TDF (%)	-	31.0 ± 0.1
SDF (%)	-	10.0 ± 0.6
IDF (%)	-	21.0 ± 0.6

NFE = nitrogen free extract; TPC = total phenolic content; TDF = total dietary fibre; SDF = soluble dietary fibre; IDF = insoluble dietary fibre.

of sugar beet powder from which the extract was obtained and was found to be 79.1%. The values of DPPH in this study are in accordance with the previous studies by Sakac *et al*. (2004). These are presented in Table 3 and Fig. 1.

Dietary fibre content in wheat-sugar beet powder cookies. Total, soluble and insoluble dietary fibre content is presented in Table 4 and graphically expressed in Fig. 2. The values for total dietary fibre content of the cookies prepared from wheat-sugar beet powder composite flour (4-20%) were ranged from 0.33-6.27%. Insoluble and soluble dietary fibre content were ranged from 0.20-4.07 and 0.13-2.20%, respectively. The highest values were shown by T_5 at 20% level and lowest by T_1 at 4% level. The values for total, soluble and insoluble dietary fibre content in different types of wheat-sugar beet powder cookies were close to the findings of Rodriguez *et al*. (2006).

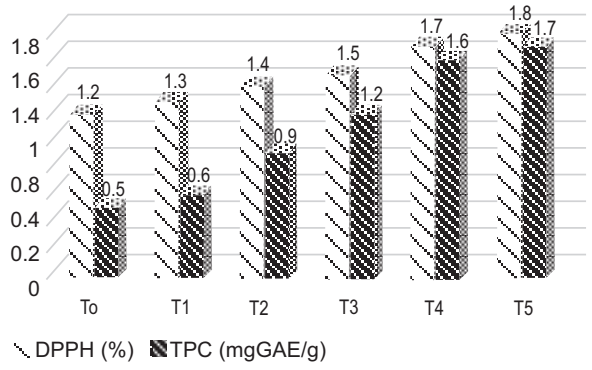

Fig. 1. Antioxidant profiling of sugar beet supplemented cookies.

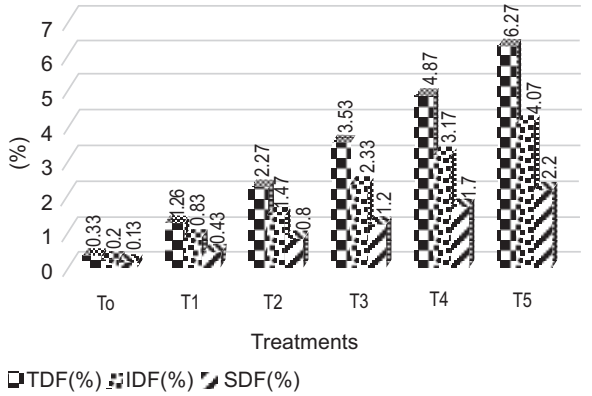

Fig. 2. Dietary fibre analysis of sugar beet supplemented cookies.

Total phenolic content and DPPH in wheat-sugar beet powder cookies. Total phenolic content and DPPH radical scavenging effect of sugar beet powder is shown in Table 3.

The values for TPC (mg GAE/g) and DPPH (%) of the cookies prepared from wheat-sugar beet powder composite flour (4-20%) were ranged from 0.6-2 mg GAE/g and 1.3-1.7%, respectively. The highest value of TPC and DPPH was shown by T_5 with a mean of 2 mg GAE/g and 1.7% at 20% level and lowest by T_1 with a mean of 0.6 mg GAE/g and 1.3% at 4% level, respectively. The values for TPC (mg GAE/g) and DPPH (%) in different types of wheat-sugar beet powder cookies are in accordance with previous study (Sakac *et al*., 2004).

Sensory evaluation of cookies. Sensory evaluation of cookies was conducted for different sensory attributes like colour, crispiness, flavour, taste, texture and overall acceptability. The sensory scores for all the above mentioned sensory attributes are shown in Table 5 and graphically expressed in Fig. 3. The scores were decreased highly significantly with increased substitution level of sugar beet powder in wheat-sugar beet powder composite flour cookies. The values of each parameter for sensorial characteristics were highest for T_3 (12%) treatment and

Table 3. Antioxidant profiling of sugar beet supplemented cookies

Treatments	DPPH (%)	TPC (mgGAE/g)
T_0	$1.2^{f} \pm 0.01$	$0.5^{f} \pm 0.1$
T_1	$1.3^{e} \pm 0.01$	$0.6^{e} \pm 0.1$
T_2	$1.4^{d} \pm 0.01$	$0.9^{d} \pm 0.1$
T_3	$1.5^{c} \pm 0.01$	$1.2^{c} \pm 0.1$
T_4	$1.6^{b} \pm 0.01$	$1.6^{b} \pm 0.1$
T_5	$1.7^{a} \pm 0.01$	$2.0^{a} \pm 0.1$

Table 4. Dietary fibre analysis of sugar beet supplemented cookies

Treatments	TDF	IDF	SDF
		(%)	
T_0	$0.33^{f} \pm 0.2$	$0.20^{f} \pm 0.1$	$0.13^{f} \pm 0.1$
T_1	$1.26^{e} \pm 0.1$	$0.83^{e} \pm 0.1$	$0.43^{e} \pm 0.1$
T_2	$2.27^{d} \pm 0.1$	$1.47^{d} \pm 0.1$	$0.80^{d} \pm 0.1$
T_3	$3.53^{c} \pm 0.1$	$2.33^{c} \pm 0.1$	$1.20^{c} \pm 0.1$
T_4	$4.87^{b} \pm 0.1$	$3.17^{b} \pm 0.1$	$1.70^{b} \pm 0.1$
T_5	$6.27^{a} \pm 0.1$	$4.07^{a} \pm 0.1$	$2.20^{a} \pm 0.1$

lowest for T_1 (4%) treatment as compared to control. Therefore, on sensory evaluation basis it was suggested that replacement of wheat flour with less than or equal to 12% sugar beet powder for cookies preparation gave the best overall acceptability of cookies and results were according to the findings of McWatters *et al*. (2003). Similarly, regarding particle size, colourless and odourless properties of these fibres have revealed better sensory characteristics of cookies (Sakac *et al*., 2010).

Table 5. Sensory evaluation of sugar beet supplemented cookies

Treatments	Colour	Crispiness	Flavour	Taste	Texture	Overall acceptability
T_0	7.2^{ab}	8.0^{a}	8.0^{a}	7.0^{ab}	8.0^{a}	7.2^{b}
T_1	7.2^{ab}	7.0^{ab}	7.0^{ab}	7.2^{a}	6.2^{bc}	7.2^{b}
T_2	6.5^{bc}	6.2^{bc}	6.2^{bc}	6.2^{b}	6.0^{bcd}	6.5^{b}
T_3	8.0^{a}	7.2^{ab}	7.8^{ab}	7.8^{a}	7.0^{ab}	8.2^{a}
T_4	5.5^{cd}	5.2^{cd}	5.2^{cd}	5.2^{c}	5.2^{cd}	5.0^{c}
T_5	4.5^{d}	4.5^{d}	4.2^{d}	4.2^{d}	4.5^{d}	4.0^{d}

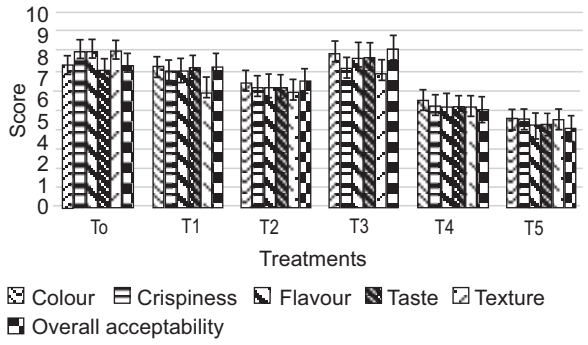

Fig. 3. Sensory evaluation of sugar beet supplemented cookies.

Conclusion

In a nutshell, sugar beet a vital source of dietary fibre provides many potent benefits. Current research has revealed that sugar beet in cookies behaves as a remarkable source of dietary fibre. The chemical composition, dietary fibre estimation both individually as well as in composite form have shown significant results and can be a good source in bakery products as cookies. Exhibits a good *in vitro* antioxidant potential including total phenolic content and DPPH by its addition in cookies. It can be recommended to be used in our routine diet in order to improve fibre intake.

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Pak. j. sci. ind. res. Ser. B: biol. sci. 2016 **59**(3) 146-154

The Influence of Storage of Pawpaw *Carica papaya* Fruit on the Bioactive Components, Antioxidative Properties and Inhibition of Fe^{2+}-Induced Lipid Peroxidation of Water-Extracts of Pawpaw Seed

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(received August 25, 2015; revised February 16, 2016; accepted March 17, 2016)

Abstract. Freshly harvested, matured, newly ripe pawpaw (*Carica papaya*) fruit (Maradol variety) was stored at room temperature (27±1 °C) for 7days. The seeds of the freshly harvested and stored pawpaw fruit were taken and divided into two groups each; one was dried as dry sample, dry freshly harvested sample (DFHS) and dry stored sample (DSS) while the other was left as wet sample, wet freshly harvested sample (WFHS) and wet stored sample (WSS). The bioactive components, antioxidative properties and inhibition of Fe^{2+} induced lipid peroxidation activity of the water extract of the seed were investigated. Storage of pawpaw fruit caused a significant increase in ascorbic acid and flavonoid content of the pawpaw seed in both dry and wet samples but only in dry stored sample significant increase in the phenol content of the seed was observed. The dried pawpaw seed DFHS and DSS showed stronger inhibition of Fe^{2+} induced lipid peroxidation activities.

Keywords: pawpaw seed, storage influence, phenol, ascorbic acid, flavonoid, DPPH scavenging ability, lipid peroxidation

Introduction

Pawpaw (*Carica papaya*) is a small tropical tree native to South America. It normally grows with a single and branched trunk which may reach 10 m in height but is more commonly 4-5 m tall. The pawpaw plant is widely spread throughout tropical Africa. It belongs to the group *Caricaceae* (Adetuyi *et al*., 2008). The skin colour of pawpaw fruit is usually green when immature, changing to fully reddish-orange or yellow-orange when fully ripened. The changes in the outer colour of the fruit skin is an indication of ripeness, and this change is considered mainly due to an increase in the carotene content and a decrease in chlorophyll (Maisarah *et al*., 2014). In the early stages of fruit development, the main sugar in pawpaw is glucose. There is an increase in sucrose content of pawpaw fruit during ripening which can be up to 80% of the total sugars. The crude protein content has been reported to range from 3.74-8.26 g/ 100 g dry matter while aspartic and glutamic acid are the most abundant amino acids in ripe fruits (Saran and Choudhary, 2013).

The central cavity of pawpaw fruit contains large quantities of seeds that are small, black, round, covered with gelatinous aril and make up about 15% of the wet weight of the fruit (Malacrida *et al*., 2011; Afolabi and Ofobrukweta, 2011). Several studies revealed that pawpaw seeds are a rich source of proteins (27.3-28.3%), lipids (28.2-30.7%), and crude fibres (19.1-22.6%). Also it contains appreciable quantities of calcium and phosphorus (Malacrida *et al*., 2011). In pawpaw seed the enzyme carpesemine is a plant growth inhibitor and oleanolic glycoside is an agent of sterility in male albino rats. Alkaloids have been reported in the endosperm of pawpaw seed. Fatty acids such as oleic, palmitic, stearic and linoleic acid have been found in pawpaw seed (Bolu *et al*., 2009). It has been discovered that glutamic acid, arginine, proline, and aspartic acid are present in the endosperm of pawpaw seed while proline, tyrosine, lysine, aspartic acid and glutamic acid are found in the sarcotesta (Saran and Choudhary, 2013).

Pawpaw latex, shoot, leaves, fruits and seeds contain a broad spectrum of phytochemicals including enzymes, carotenoids, alkaloids, phenolics, and glucosinolates. People in Lao, Cambodia, and Vietnam use the latex to treat eczema and psoriasis. The seeds have been used as vermifuge, thirst quencher, or pain alleviator (Krishna *et al*., 2008; Amenta *et al*., 2000). Phenolic compounds

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in pawpaw fruit peel and seeds have antioxidative properties (Ang *et al*., 2012). The seed of pawpaw has antimicrobial activity against *Trichomonas vaginalis* trophozoites. The seeds, irrespective of its fruit maturity stages have bacteriostatic activity on gram positive and gram negative organisms which could be useful in treating chronic skin ulcer (Afolabi and Ofobrukweta, 2011).

Pawpaw seed has shown clearly to have antihelminthic, immunomodulatory activities and having anti-amoebic properties; they are also rich source of amino acids especially in the sarcotesta. Air dried papaya seeds with honey showed significant effect on human intestinal parasites without significant side effect (Saran and Choudhary, 2013; Okeniyi *et al*., 2007). The pawpaw seed extract has shown to possess gastro-protective effect on ethanol induced gastric ulcer in rats and also used in treatment of sickle cell disease (Saran and Choudhary, 2013; Okewumi and Oyeyemi, 2012). Seeds of overripe pawpaw fruit are good source of protein, energy, nutritive minerals and have low antinutrient values (Oyeleke *et al*., 2013).

There has been no previous study to directly compare bioactive contents and antioxidant properties of pawpaw seed, with respect to storage of pawpaw fruit. Therefore, the aim of this study was to determine the effect of storage of pawpaw fruit at room temperature on the bioactive content and antioxidant properties of pawpaw seed.

Materials and Methods

Materials. The maturity stages of the pawpaw used in this study were evaluated visually based on the skin colour as defined by Hana and Angelo (2006): Stage 0 - totally green; Stage 1 - yellow colour that does not cover more than 15% of skin surface; Stage 2 - fruit with 16-25% of yellow skin; Stage 3 - fruit with 26-50% of yellow skin. Freshly harvested, matured, newly ripe pawpaw fruit (Maradol variety) stage 1 with slight appearance of yellow colour obtained from Ondo State University of Science and Technology farm was used for this study.

Reagents. Ascorbic acid was from Merck (Darmstadt, Germany), gallic acid and quercetin were from Aldrich (Steinheim Germany), 1,1-diphenyl-2 picrylhydrazyl (DPPH), 1,10 orthophenanthroline, trichloroacetic acid (TCA) and potassium ferricyanide were obtained from Sigma Chemical Inc. (St Louis, MO, USA). Glass-distilled water and analytical grade chemicals and solvent were used in the present study.

Sample preparation. The freshly harvested pawpaw fruit was stored at room temperature (27±1 ºC) for 7 days. The seed of the freshly harvested and stored pawpaw fruit (200 g each) were taken and divided into two groups; one was dried in the oven at 45 °C for 5 days as dry sample, dry freshly harvested sample (DFHS) and dry stored sample (DSS) while the other was left as wet sample, wet freshly harvested sample (WFHS) and wet stored sample (WSS). The ascorbic acid, total phenol, flavonoid, ABTS scavenging ability, ferric reducing antioxidant property (FRAP), Fe^{2+} chelation assay, DPPH free radical scavenging ability and lipid peroxidation of the dry and wet samples were determined. All determinations were done in triplicates.

Aqueous extract preparation. The aqueous extracts of pawpaw seeds was prepared using a modified procedure described by Oboh *et al*. (2010). Ten grams (10 g) of the dry pawpaw seed and wet pawpaw seed were separately homogenised in 100 mL distilled water in a Warring blender. The homogenate was centrifuged at 4500 g for 15 min. The supernatant was used for the determination of the bioactive content and antioxidant properties of pawpaw seed.

Ascorbic acid content determination. The ascorbic acid content of the aqueous extract was determined using the method of Benderitter *et al*. (1998). 75 µL DNPH (2 g dinitrophenyl hydrazine, 230 mg thiourea and 270 mg $CuSO_4$. $5H_2O$ in 100 mL of 5 mL H_2SO_4) was added to 500 µL extracts mixture (300 µL of an appropriate dilution of the extract with 100 µL 13.3% trichloroacetic acid (TCA and water). The reaction mixture was subsequently incubated for 3 h at 37 °C, then 0.5 mL of 65% H_2SO_4 (v/v) was added to the medium and the absorbance was measured at 520 nm in a spectrophotometer (JENWAY 6305). The vitamin C content of the extracts was subsequently calculated using ascorbic acid as standard.

Phenolic content determination. The total phenol content was determined according to the method of Singleton *et al*. (1999). Appropriate dilutions of the extracts were mixed with 2.5 mL of 10% Folin-Ciocalteau's reagent (v/v) and neutralised by 2.0 mL of 7.5% sodium carbonate. The reaction mixture was incubated for 40 min at 45 °C and the absorbance was measured at 765 nm in a spectrophotometer (JENWAY 6305). The total phenol content was subsequently calculated using gallic acid as standard.

Flavonoid content determination. The total flavonoid content of the extracts was determined using a slightly modified method reported by Meda *et al*. (2005). Briefly, 0.5 mL of appropriately diluted sample was mixed with 0.5 mL methanol, 50 mL of 10% aluminium chloride ($AlCl_3$), 50 mL of 1 mol/L potassium acetate and 1.4 mL water, and allowed to incubate at room temperature for 30 min. Thereafter, the absorbance of the reaction mixture was subsequently measured at 415 nm using a spectrophotometer (JENWAY 6305). The total flavonoid was calculated using quercetin as standard.

Ferric reducing antioxidant power (FRAP). The reducing property of the extracts was determined by assessing the ability of the extract to reduce $FeCl_3$, solution as described by Oyaizu (1986). A 2.5 mL aliquot was mixed with 2.5 mL of 200 mM sodium phosphate buffer (pH 6.6) and 2.5 mL of 1% potassium ferricyanide. The mixture was incubated at 50 °C for 20 min; thereafter 2.5 mL of 10% trichloroacetic acid was added. This mixture was centrifuged at 2000 g for 10 min; 5 mL of the supernatant was mixed with an equal volume of water and 1 mL of 0.1% ferric chloride. The absorbance was measured at 700 nm in a spectrophotometer (JENWAY 6305) and ferric reducing antioxidant property was subsequently calculated using ascorbic acid as standard.

Iron chelating activity. Iron chelating activity was determined according to the method of Kuda *et al*. (2005). The pawpaw sample solution (0.1 mL) was taken into 10 mL test tube, 0.1 mL of distilled water and 0.025 mL of 2.5 mM iron chloride were added, it was thoroughly shaken and the absorbance was taken at 550 nm (Abs1). 0.025 mL of 2.5 mM ferrozine was added to the mixture, it was allowed to stay for 20 min at room temperature; the absorbance was taken at 550 nm (Abs2).

DPPH free radical scavenging ability. The free radical scavenging ability of the extract was determined using 1, 1-diphenyl-2-picryl hydrazyl (DPPH) as described by Singh *et al*. (2002). Different concentrations of the aqueous extract were taken in different test tubes and the volume was prepared to 1 mL with distilled water. 4 mL of 0.1 mM methanolic solution of DPPH was added. The tubes were shaken vigorously and allowed to stand for 20 min at room temperature. A control was prepared as above without the sample and distilled water was used for base line correction. Changes in absorbance of samples were measured at 517 nm in a spectrophotometer (JENWAY 6305). Free radical scavenging activity was expressed as percentage inhibition and was calculated using the following formula:

Free radical scavenging activity (%) = (control OD – sample OD)/control OD×100

ABTS scavenging ability. The ABTS scavenging ability was determined according to the method described by Re *et al*. (1999). The ABTS was generated by reacting an ABTS aqueous solution (7 mM/L) with potassium persulphate ($K_2S_2O_8$) (2.45 mM/L, final concentration) in the dark at room temperature for 16 h and adjusting the absorbance at 734 nm to 0.700 with ethanol. 0.2 mL of appropriate dilution of the extract was added to 2.0 mL ABTS solution and the absorbance was measured at 734 nm after 15 min in the JENWAY uv - visible spectrophotometer. The trolox equivalent antioxidant capacity was subsequently calculated.

Lipid peroxidation assay. ***Preparation of tissue homogenates*.** The rats (12 weeks old and weighing between 220 and 240 g) were decapitated under mild diethyl ether anaesthesia and the whole brain rapidly isolated and placed on ice and weighed. This tissue was subsequently homogenized in cold saline (1:10, w/v) with about 10 up and down strokes at approximately 1200 rpm in a Teflon glass homogenizer. The homogenate was centrifuged for 10 mins at 3000 × g to yield a pellet that was discarded, and a low – speed supernatant (SI) which was kept for lipid peroxidation assay (Belle *et al*., 2004).

Lipid peroxidation and TBA reactions. The lipid peroxidation assay was carried out using the modified method of Ohkawa *et al*. (1979). Briefly, 100 µL SI fraction was mixed with a reaction mixture containing 30 µL of 0.1M Tris-HCl buffer (pH 7.4), the extract (0 - 100 µL) and 30 µL of 250 µM freshly prepared $FeSO_4$. The volume was made up to 300 µL by water before incubation at 37 °C for 1 h. The colour reaction was developed by adding 300 µL of 8.1% sodium dodecyl sulphate (SDS) to the reaction mixture containing SI; this was subsequently followed by the addition of 600 µL acetic acid/HCl (pH 3.4) mixture and 600 µL of 0.8% thiobarbituric acid (TBA). This mixture was incubated at 100 °C for 1 h. Thiobarbituric acid reactive species (TBARS) produced were measured at 532 nm in the JENWAY UV-visible spectrophotometer and the absorbance was compared with that of standard curve using malondialdehyde (MDA).

Statistical analysis. The results of the three replicates were pooled and expressed as mean ± standard deviation. Standard deviations were calculated using spread sheet software (Microsoft Excel©, version 2013). Analysis of variance (ANOVA) was performed using Statistical Analysis System proprietary software (SAS, 2002). Duncan's multiple range test procedure as described in the SAS software was used for mean separations. Significance was accepted at P = 0.05. IC_{50} (extract concentration causing 50% inhibition of antioxidant activity) was calculated using the linear regression analysis.

Results and Discussion

Ascorbic acid. The ascorbic acid, total phenol and flavonoid contents of the extract of pawpaw seed are presented in Table 1. The ascorbic acid content of the extract ranged from 27.14 mg ascorbic acid equivalent (AAE/100 g) wet freshly harvested sample (WFHS) to 37.14 mg AAE/100g wet stored sample (WSS). The value of ascorbic acid reported in this work was higher than that reported for papaya seed but lower to the ascorbic acid of papaya pulp and peel (Maisarah *et al*., 2014; Santos *et al*., 2014; Nwofia *et al*., 2012). The result showed that the ascorbic acid content of wet sample extracts (WFHS and WSS) was significantly higher than the corresponding dry sample extracts (DFHS and DSS). This could be because ascorbic acid is easily destroyed by heat. The ascorbic acid content of the seed of stored pawpaw fruit in both wet and dry samples was significantly higher than the seed of freshly harvested fruit. The high ascorbic acid content of the seed of stored pawpaw fruit could be as a result of ripening since the pawpaw fruit stored at room temperature has ripened more than the freshly harvested pawpaw fruit. Ripening has been observed to cause increase in vitamin C content of guava and mango (Ortega *et al*., 2013; Gull *et al*., 2012). The increase in ascorbic acid contents of the seed of stored pawpaw fruit as a result of ripening may be due to physiological activities that persist after harvesting of the fruit which is the breakdown of starch to glucose and this causes increase in the biosynthesis of ascorbic acid. The increase in ascorbic acid content during ripening has also been attributed to the increase in lipid peroxidation which is another physiological activity going on in the ripened fruit (Jimenez *et al*., 2002).

Phenolic content. The levels of total phenol content in the seed of the pawpaw fruit varied significantly from 25.24 to 41.43 mg gallic acid equivalent (GAE/g). The dry seed of stored pawpaw fruit DSS contained the highest phenolic content (41.43 mg GAE/g), followed by the dry seed of freshly harvested pawpaw fruit DFHS (34.29 mg GAE/g), wet seed of freshly harvested pawpaw fruit WFHS (25.71 mg GAE/g) and wet seed of stored pawpaw fruit WSS (25.24 mg GAE/g). The phenolic content reported in this work was higher than the value reported in previous study for papaya seed on dry weight basis (Maisarah *et al*., 2014; Santos *et al*., 2014) but lower to 211.31 g GAE/g and 156.53 g GAE/g reported for water and hexane extract of papaya seed (Kothari and Seshadri, 2010). The result indicated that the dry seed of pawpaw fruit (DFHS and DSS) contain high phenolic content in both freshly harvested and stored pawpaw fruit, which may provide good sources of dietary antioxidant and gastro protective medicine. The phenolic content of the wet samples of pawpaw seed decreased in storage but not significant. The stored pawpaw fruit has ripened in storage than freshly harvested which could be a factor for the decrease in the phenolic content. The findings of Gull *et al*. (2012) in ripening stages of guava also showed that there was an apparent gradual decrease in the total phenolic contents during ripening of guava fruit, which might be associated with an amplified polyphenol oxidase activity. Ripening and other physiological activities continue in all plant crops following harvesting. These processes involve changes in the chemical composition and physical characteristics of the plant material and can influence its quality as food, whether it is consumed fresh or used as raw material for subse-quent processing operations (Rhodes, 1980).

Table 1. Phenol, flavonoid, nonflavonoid and ascorbic acid content of pawpaw seed

Pawpaw sample	Phenol (mg/GAE/g)	Flavonoid (mg/QE/g)	Non flavonoid (mg/QE/g)	Ascorbic acid (mg/AAE/ 100 g)
WFHS	25.71 ± 0.00^{c}	3.16 ± 0.00^{d}	22.55 ± 2.33^{c}	35.72 ± 2.02^{b}
DFHS	34.29 ± 0.00^{b}	5.79 ± 0.00^{b}	28.50 ± 1.89^{b}	27.14 ± 6.05^{d}
WSS	25.24 ± 0.67^{c}	3.95 ± 0.36^{c}	21.29 ± 0.00^{d}	37.14 ± 0.00^{a}
DSS	41.43 ± 0.67^{a}	7.63 ± 0.37^{a}	33.80 ± 2.17^{a}	34.29 ± 0.00^{c}

Values represent mean ± standard deviation of triplicate determination. Values with the same letter along the same column are not significantly different ($P \leq 0.05$). GAE = gallic acid equivalent; AAE = ascorbic acid equivalent; QE = quercetin equivalent; WFHS = wet freshly harvested sample, DFHS = dry freshly harvested sample, WSS = wet stored sample, DSS = dry stored sample.

Flavonoid content. The result of Flavonoid in mg quercetin equivalent (QE/g) revealed that dry seed of pawpaw fruit at both freshly harvested and stored fruit had a significantly higher flavonoid (7.63 mg QE/g (DSS) and 5.79 mg QE/g (DFHS) content than wet seed of pawpaw fruit (3.95 mg QE/g (WSS) and 3.16 mg QE/g (WFHS)). The flavonoid in the present study was within the range of values reported by Kothari and Seshadri (2010) for papaya seed using ethanol and water extraction method 10.20-4.42 mg QE/g but higher than 59.54 mg/100 g reported by Maisarah *et al*. (2013). There was an increase in the flavonoid content of pawpaw seed due to storage. The basis of the increase could not be categorically stated; though, it may be due to ripening, a physiological activity that releases ethylene, a phytohormone that stimulates the activity of the key enzyme involved in polyphenol biosynthesis, phenylalanine ammonium lyase (PAL), which leads to formation of polyphenols. PAL activity has been shown to have a direct influence on total flavonoids (Matthes and Schmitz-Eiberger, 2009). An increase in polyphenol content during shelf life has been explained to be caused by a higher ethylene production which resulted in a stimulation of PAL (Napolitano *et al*., 2004). The non-flavonoid content of the pawpaw seed of freshly harvested pawpaw fruit and stored pawpaw fruit followed the same observation for both the total phenol and flavonoid contents where the values for the dry seeds of pawpaw fruit were significantly higher than others in the order of DSS > DFHS > WFHS > WSS (Table 1).

In complex systems, like food and food preparations, there are different mechanisms that may contribute to oxidative processes, in some mechanism, transition metal ions play a vital role, where different ROS may be generated and molecules like lipids, proteins and carbohydrates may be affected, while in another mechanism the ability of the extracts to donate electrons or the ability to protect carbohydrate structures or the ability to chelate Fe^{2+} is tested (Hinneburg *et al*., 2006; Halliwell, 1997). Several food components like carotenoids, vitamin C and E, polyphenolic compounds and the synergisms among them contribute to the overall antioxidant ability of food, it will then be difficult to assess total antioxidant ability on the basis of these individual active components alone (Pinelo *et al*., 2004). It is on this premise that ferric reducing antioxidant power (FRAP), iron chelating activity, 1,1-diphenyl-2 picrylhydrazyl (DPPH) radical scavenging abilities, 2,2-azinobis-3-ethylbenzo-thiazoline-6-sulphonate (ABTS), lipid peroxidation and thiobarbibutric acid reactions (TBARS) were evaluated for the measurement of total antioxidant activity of pawpaw seed in both freshly harvested and stored pawpaw fruit.

Ferric reducing antioxidant power (FRAP). Iron (III) ion Fe^{3+} reduction has been used many times as an indicator of electron- donating activity, which is an important mechanism of phenolic antioxidant action (Hinneburg *et al*., 2006). The Fe^{3+} to Fe^{2+} reducing activity of the pawpaw seed is expressed as mg Ascorbic Acid Equivalents AAE/g sample. Figure 1 shows that all the extracts have the ability to reduce Fe^{3+} to Fe^{2+}. The best activity was found in dry seed of stored pawpaw fruit DSS (14.28 mg AAE/g) which was significantly higher than the other extracts. There was no significant difference in the FRAP activity of the wet seed extract of freshly harvested pawpaw fruit WFHS and wet seed extract of stored pawpaw fruit WSS, the wet seed extracts performed significantly lower than the dry seed extracts in both the freshly harvested and stored pawpaw fruit. The reducing power of pawpaw seed extracts from the FRAP results demonstrated a potential antioxidant activity in consumer's body. Such a remarkable high reducing power in the dry seed of stored pawpaw fruit (DSS) was attributed to their high phenolic and flavonoid content. FRAP assay has been considered to be a good

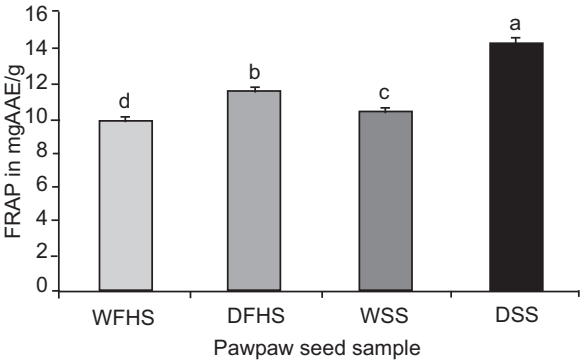

Fig. 1. Ferric reducing antioxidant property (FRAP) of pawpaw seed.
Values represent mean ± standard deviation of triplicate determination. Values with the same letter are not significantly different ($P \leq 0.05$). AAE – Ascorbic Acid. WFHS = wet freshly harvested sample, DFHS = dry freshly harvested sample, WSS = wet stored sample, DSS = dry stored sample.

method in evaluating the antioxidant capacity of fruits (Palafox-Carlos *et al*., 2012). However, it was discovered that FRAP have some disadvantages in that any electron-donating substances with redox potential lower than that of the redox pair Fe^{3+}/Fe^{2+} can contribute to the FRAP value and indicate a false high values of FRAP even if they don't have antioxidant properties (Nilsson *et al*., 2005).

Iron chelation. Foods are always contaminated with transition metal ions and these may be introduced by processing methods. Bivalent transition metal ions have a crucial role to play as catalysts of oxidative processes, leading to the formation of hydroxyl radicals and hydroperoxide decomposition reactions via Fenton chemistry (Halliwell, 1997). These can be prevented by iron chelation and deactivation. Therefore, the ability of the extracts of the pawpaw seed to chelate Fe^{2+} ions was evaluated and results are presented in Table 2. The seed of stored pawpaw fruit in the dry sample (DSS) and wet sample (WSS) showed the best iron chelating ability of 25.65%, respectively, which was significantly higher than others; it was closely followed by the dry seed of freshly harvested pawpaw fruit (24.36%). The basis for the high chelating ability of WSS 25.65% could not be categorically stated; however, it is expected that DSS would have a high chelation activity because of its high phenolic and flavonoid content which has been positively correlated to the iron chelation activity of food (Al-Farga *et al*., 2014; Hinneburg *et al*., 2006).

The DPPH. The DPPH results were used to confirm the results obtained in total phenolic content (Palafox-Carlosa *et al*., 2012). DPPH radical-scavenging ability of the pawpaw seed at 0-33.33 mg/mL concentrations was measured and the results are presented in Fig. 2. A dose-response relationship was found in the DPPH radical-scavenging ability of the pawpaw seed extracts; the ability increased significantly with an increase in the concentration of the pawpaw seed extracts. It showed from these results that the dry seed of pawpaw fruit in both freshly harvested and stored pawpaw fruit (DSS and DFHS) recorded the highest DPPH radical-scavenging ability which was significantly higher than the wet seed of pawpaw fruit (WFHS and WSS). It can be seen from Table 2 that DSS with the lowest IC_{50} (15.08 mg/mL) exhibited the highest DPPH radical-scavenging activity, followed by DFHS (IC_{50} 15.36 mg/mL). These result suggested that dry pawpaw seed (DSS and DFHS) are competent DPPH radical scavenger. This is an indication that it has the ability to protect consumer's health from various free-radical related diseases including ageing, cancer, atherosclerosis, Alzheimer disease, diabetes and so on.

Table 2. Fe^{2+} chelation, ABTS, IC_{50} of DPPH and lipid peroxidation of pawpaw seed

Pawpaw sample	Fe^{2+} chelation (%)	ABTS (mmol.TEAC/ 100 g)	DPPH IC_{50}(mg/ mL)	Lipid peroxidation IC_{50}(mg/mL)
WFHS	21.80±5.43^{c}	2.08±0.27^{c}	33.66±4.12^{b}	11.28±1.00^{a}
DFHS	24.36±1.81^{b}	7.17±0.53^{a}	15.36±1.81^{d}	4.5±0.00^{d}
WSS	25.65±3.62^{a}	0.94±0.26^{d}	38.60±3.76^{a}	6.53±0.64^{b}
DSS	25.65±3.63^{a}	6.04±0.53^{b}	15.08±1.67^{c}	5.68±0.63^{c}

Values represent mean ± standard deviation of triplicate determination. Values with the same letter along the same column are not significantly different ($P \leq 0.05$). TEAC = trolox equivalent antioxidant capacity; WFHS = wet freshly harvested sample; DFHS = dry freshly harvested sample; WSS = wet stored sample; DSS = dry stored sample.

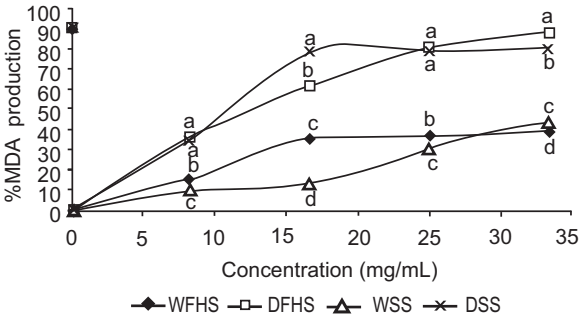

Fig. 2. DPPH scavenging abilities of pawpaw seed. Values represent mean ± standard deviation of triplicate determination. Values with the same letter along the same column are not significantly different ($P \leq 0.05$). WFHS = wet freshly harvested sample, DFHS = dry freshly harvested sample, WSS = wet stored sample, DSS = dry stored sample.

The ABTS. The ABTS radical has been used to confirm results obtained with DPPH, because both possess similar antioxidant mechanisms. The versatility of ABTS radical scavenging ability is an advantage since it can measure both non-polar and polar antioxidants and spectral interference is minimized as the absorption maximum used is 760 nm, a wavelength not normally encountered with natural products (Perez-Jimenez *et al*., 2008; Re *et al*., 1999).

ABTS scavenging ability reported as trolox equivalent antioxidant capacity (TEAC) of the pawpaw seed of freshly harvested pawpaw fruit and stored pawpaw fruit is presented in Table 2. The result revealed that the ABTS scavenging ability of dry seed of freshly harvested pawpaw fruit DFHS 7.17 mmol. TEAC/100 g was significantly higher than other samples, closely followed by the dry seed of stored pawpaw fruit DSS 6.04 mmol. TEAC/100 g. The higher the TEAC value of the sample, the stronger the antioxidant activity (Zarena and Sankar, 2009). It is to be noted that high antioxidant activities in extracts may be attributed to the phenolic and flavonoid content of the extract, it was expected considering the high phenolic and flavonoid content of the extract of dry seed of stored pawpaw fruit (DSS) to have the highest ABTS scavenging ability but contrary is the case in this work where ABTS scavenging ability observed do not follow the order of phenolic and flavonoid content.

Lipid peroxidation. The polyunsaturated fatty acids (PUFAs) rich phospholipids in the brain membranes are easily attacked by free radicals, causing oxidative damage to the brain phospholipids which could result to the development of Alzhemier's disease (Axelsen *et al*., 2011). The protective capacity of the pawpaw seed extracts against Fe^{2+} induced lipid peroxidation in cultured rat brain, is shown in Fig. 3. Incubation of rat's brain in the presence of 250 µM iron (II) caused a significant increase in the malondialdehyde (MDA) content of the brain (180%). The increase in iron in this organ resulted in the formation of reactive oxygen species ROS which facilitates lipid peroxidation through Fenton reaction. This could have accounted for the increase in MDA contents of the brain after incubation in the presence of Fe^{2+}. The result showed that the aqueous extracts of pawpaw seed significantly inhibited MDA production in the brain in a dose dependent manner. The dry seed of freshly harvested pawpaw fruit and stored pawpaw fruit had the highest inhibitory effect on the Fe^{2+} induced lipid peroxidation in rat brain since they exhibited the least IC_{50} 4.05 mg/mL DFHS and 5.68 mg/mL DSS (Table 2). The lipid peroxidation inhibition in this work followed the trend observed for ABTS scavenging ability where the scavenging ability of the dry seed of freshly harvested pawpaw fruit (DFHS) was significantly higher than other samples, closely followed by the dry seed of stored pawpaw fruit (DSS). The possible mechanism through which the extracts protect the brain could be by Fe^{2+} chelation and the scavenging of hydroxyl ion (Ademosun and Oboh, 2014). The ability of pawpaw seed extracts to inhibit Fe^{2+} induced lipid peroxidation in rat brain homogenate *in-vitro* is a reflection that pawpaw seed extracts could prevent the elevation of brain iron levels which can cause increase in iron transport across the blood brain barrier and resulted to extracellular iron overload of the brain that can lead to neurodegenerative condition of Alzhemier's disease.

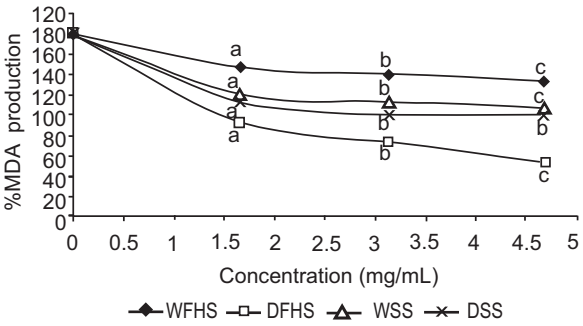

Fig. 3. Inhibition of Fe^{2+} induced MDA production in rat brain by pawpaw seed extract.
Values represent mean ± standard deviation of triplicate determination. Values with the same letter along the same column are not significantly different ($P \leq 0.05$). WFHS = wet freshly harvested sample, DFHS = dry freshly harvested sample, WSS = wet stored sample, DSS = dry stored sample.

Conclusion

The total phenol and flavonoid content of pawpaw seed in dry samples increased when pawpaw fruit was stored but storage of pawpaw fruit has no significant effect on the total phenol and flavonoid content of pawpaw seed in wet samples. Storage of pawpaw fruit caused an increase in the ascorbic acid content of pawpaw seed in both wet and dry samples. Storage of pawpaw fruit also caused an increase in the scavenging abilities of dry pawpaw seed since the dry pawpaw seed of stored pawpaw fruit exhibited higher antioxidant activities. The knowledge about the antioxidant composition of the seeds of stored pawpaw fruit when dried provides important information for pharmaceutical and food industries interested in adding value to pawpaw fruit.

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Pak. j. sci. ind. res. Ser. B: biol. sci. 2016 **59**(3) 155-163

Stabilisation of Some Vegetable Oils by Sugarcane Leaf Extract at Ambient Temperature

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(received February 19, 2015; revised October 10, 2015; accepted November 13, 2015)

Abstract. The present study was aimed to assess the antioxidant activity of ethanolic sugarcane leaf extract for the stabilisation of sunflower, (SFO), soybean and (SBO) canola oils (CO) at ambient temperature. SFO, SBO and CO were added with 600 ppm sugarcane leaf extract, filled in transparent PET bottles, stored at ambient temperature for 180 days, sampled at 0, 60, 120 and 180 days for the assessment of oxidative stability. Total phenolic content in sugarcane leaf extract (SLE) was 724.3 (mg GAE/100g). 2,2,diphenyl- 2 picrylhydrazyl free radical scavenging activity of SLE was 76% as compared to 88% in butylated hydroxyl toluene. C18:1 and C18:2 in fresh, 6 months stored controls and SLE supplemented SFO were 46.12%, 42.59%, 44.91% and 47.15%, 40.29, 43.13%, respectively. C18:2 and C18:3 in fresh and 180 days stored control and SLE supplemented SBO were 51.19%, 45.61%, 48.97% and 6.19%, 3.37% and 5.67%, respectively. Similar trend was also recorded in canola oil. Induction period of supplemented vegetable oil was higher than the un-supplemented samples ($P<0.05$). Viscosity and specific gravity of supplemented vegetable oils were not different from non-supplemented samples. Sensory characteristics of SLE supplemented vegetable oils were not different from the control. Sugarcane leaf extract can be used for the long term preservation of SFO, SBO and CO at ambient temperature.

Keywords: sunflower oil, soybean oil, canola oil, oxidative stability, sugarcane leaf

Introduction

The auto-oxidation and photo-oxidation susceptibility of vegetable oils is one of the biggest problems of vegetable oil processing industry (Choe and Min, 2006). Health apprehensions of rancid vegetable oils are even worse than bad fats because of the absorption of several oxidation products in the body (Quiles *et al*., 2002). Auto-oxidation of fats and oils leads to the generation of potentially toxic oxidation products which have been connected in a large number of health related disparities such as atherosclerosis, carcinogenesis etc. (Turner *et al*., 2006). The conversion of primary oxidation products into highly reactive and toxigenic secondary oxidation products such as 4-hydroxy-2-alkenals has led to the great deal of concerns regarding the consumption of oxidized fats, the toxicity of oxidation products for L6 muscles has also been scientifically proven (Pillon *et al*., 2010; Zarate *et al*., 2009). Further, the nutritional and sensory characteristics of oxidized fats and oils are on the lower side due to the generation of a wide range of disagreeable flavouring compounds, loss of fats soluble vitamins and essential fatty acids (Kanner, 2007). Degree of unsaturation, processing conditions, metal ions, antioxidants, light and heat greatly influence the oxidation of vegetable oils. The undesirable activities of reactive oxygen species (such as superoxide, hydrogen peroxide and hydroxyl radicals) can cause oxidative damage to the cells, the unrestrained free radicals in body can lead to the damage of DNA, lipids, proteins, lipoproteins, carcinogenesis, cardiovas-cular diseases, diabetes, stroke and accelerated ageing (Adedapo *et al*., 2008;Yazdanparast and Ardestano, 2007). The role of phytochemicals as biologically active compounds in the neutralisation of free radicals, protective against oxidative damage and inhibition of lipid peroxidation has been well established (Valantina *et al*., 2009).

Sugarcane (*Sacchrum officinarum* L.) is one of the most widely cultivated cash crops in the world and recent studies have reported the presence of phenolic acids, flavonoids with diuretic, anti-anaemic, anti-ulcer and live protective properties. Although some work has been previously conducted to assess the antioxidant characteristics of sugarcane leaves, the detailed work is the need of hour to explore its antioxidant potential for the stabilisation of some commercially important vegetable oils. The oxidative stabilisation of vegetable oils through natural antioxidants has been extensively studied (Nadeem *et al.,* 2014) but the antioxidant activity

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of sugarcane leaf extract for the stabilisation of sunflower (SFO), soybean (SBO) and canola (CO) has not been previously investigated. This study aimed to stabilise SFO, SBO and CO through ethanolic sugarcane leaf extract at room temperature by the assessment of oxidative stability through some conventional and advanced analytical techniques.

Materials and Methods

Refined, bleached and deodorized (RBD) sunflower, soybean and canola oils (SFO, SBO and CO) without any additives were obtained from United Industries Ltd. Kashmir Road, Nishatabad, Faisalabad, Pakistan and their chemical camposition was noted (Table 1). Sugarcane leaves were obtained from Ayub Agricultural Research Institute, Faisalabad. All the reagents used in this work were HPLC grade and obtained from Sigma Aldrich, UK.

Preparation of antioxidant extract. Fresh leaves of surgarcane were washed with tap water, dried in the shade and ground to 100 mesh size. The sample (20 g) was mixed with 100 mL ethanol in a Pyrex beaker, mixed at 200 rpm for 8 h at room temperature (20-22 °C), filtered over Whatman filter paper and the residue was again extracted in a similar manner, pooled, evaporated on a rotary evaporator at 45°C (Buchi, Japan) till 10 mL, preserved in amber bottles and stored at 65 °C (Sanyo) till further use in this study.

Experimental plan. SFO, SBO and CO were added with 600 ppm sugarcane leaf extract, filled in transparent PET bottles, stored at ambient temperature for 180 days, sampled at 0, 60, 120 and 180 days for the assessment of oxidative stability.

Total phenolic contents. For the determination of total phenolic contents 5 mL Folin-ciolcalteu and 10 mL distilled water were mixed then 5 mL of this solution was mixed with 1 mL extract and sodium carbonate (7.5%), vortexed at 2200 rpm for 2 min and incubated at room temperature for the development of colour for 1 h and measured on a double beam spectrophotometer at 760 nm (Shimadzu, Japan). The concentration of total phenolic contents were calculated from the calibration curve (R^2=0.994) and expressed in terms of GAE/ 100 g (Wolf *et al*., 2003).

Determination of antioxidant activity in linoleic acid system. To a solution containing 0.13 mL linoleic acid, 10 mL 99.8% ethanol and 7 mL phosphate buffer (0.2 M; pH 7), 5 mg SLE was added, distilled water was added to make the volume of mixture to 25 mL, incubated at 4 °C. The extent of oxidation was determined as per thiocyanate method, 10 mL 75% ethanol, 30% ammonium thiocyante (0.2 mL), 0.2 mL SLE and 0.2 mL ferrous chloride solution (20 mL in 3.5% HCl) were added in a sequence. The absorption was read at 500 nm after 3min of stirring and compared with a control (without SLE). Butytated hydroxyl anisole (BHA) and butylated hydroxyl toluene (BHT) were employed as positive controls. The inhibition of linoleic acid peroxidation was calculated by the following expression to assess the antioxidant activity of extract (Yen and Duh, 1993).

100 – [(Absorbance of sample at 336 h/ absorbance of control at 336 h)]

DPPH free radical scavenging activity. DPPH solution (2,2,diphenyl-2 picrylhydrazyl) 1.95 (6×10^{-5}; 50 µL) was mixed with sugarcane leaf extract at different concentrations, left at room temperature for 60 min and absorbance was measured on double beam spectrophotometer at 515 nm (Mansouri *et al*., 2005). DPPH free radical scavenging activity was calculated by the following expression

% DPPH free radical scavenging activity= (AB-AS)/ AB × 100

where:

AB and AS is the absorbance of blank and sample, respectively.

Experimental plan. Sugarcane leaf extract (SLE) was incorporated into RBD SFO (winterized), SBO and CO at 600 ppm concentration, compared with the controls (SFO, SBO and CO without SLE as positive controls). Filled in PET bottles, stored at ambient temperature and oxidative stability was measured at 0, 60, 120 and 180 days of storage period.

Analysis. Peroxide, anisidine and iodine values were determined by the standard methods (AOCS, 1995). Specific extinctions at 232 and 280 nm were measured on a double beam spectrophotometer (Shimadzu, Japan) as per method (IUPAC, 2006). Two methods were used to assess the antioxidant potential of sugarcane leaf extract in the accelerated oxidation conditions; in the first method Schaal oven test was performed by exposing the vegetable oils added with SLE and controls to 63°C for 5 days, extent of peroxide value was determined as an adequacy of the antioxidant capacity of SLE (AOCS, 1995). Induction period was determined on a Rancimat 679 as per instructions prescribed in the instruction

manual of Metrohm Corporation, Switzerland (Metrohm, 1993). Colour was checked in 5.25″ quartz cell (Lovibond Tintometer Salisbury, England). The fatty acid methyl esters were prepared by sodium methoxide trans esterification technique in iso-octane, the supernatant FAME layer was injected at a 1µL concentration into the GC (Perkin Elmer Instrument, Auto system XL) using SP-2380 fused silica capillary column (30 m × 0.25 mm Supelco Bellefonte, PA) using nitrogen (1.5 mL/min) as a carrier gas (Qian, 2010). Fatty acids were identified and quantified by using FAME Mix GLC-30, Supelco). The results of triplicate treatments and three time analysis of each samples were expressed as Mean±SD and Duncan Multiple Range Test was used to determine the significant difference among the treatments (Steel *et al*., 1997).

Results and Discussion

Total phenolic contents. Total phenolic contents in ethanolic extract of SLE were 724.3 (mg GAE/100 g). Souza-Sartori *et al*. (2013) observed that the concentration of flavonoids in sugarcane leaf extract was 50.98 mg/100 mL. The antioxidant activity of sugarcane leaves is due to luteolin-8-C-(rhamnosyl glucoside) (Fabiana *et al*., 2008). The beneficial effects of polyphenols as antimicrobial, antimutagenic, antiproliferative, and vasodilatory activities has been well established *in vitro* studies (Lazarou *et al*., 2007; Taguri *et al*., 2006). Polyphenols can prevent tooth decay by inhibiting the activities of *Streptococcus mutans*, improve memory and lack of concentration disorders (Magdalini *et al*., 2009; Sasaki *et al*., 2004). The strong antioxidant activity of methanolic extract of sugarcane leaves has also been reported in Chinese sugarcane. In current investigation, the ethanolic extract of sugarcane top leaves also revealed the higher extent of phenolics. The literature suggest the better antioxidant activity of plant based antioxidants, but from the safety view point methanolic extracts for the stabilisation of food substrates could have a high degree of concerns due to its toxicity. Abbas *et al*. (2013) reported a great deal of neutralization of free radicals by the leaves of sugarcane from seven different cultivars of Pakistan.

DPPH free radical scavenging activity. DPPH free radical scavenging activity is a useful assay to determine the antioxidant activity of antioxidants. The scavenging of DPPH is based on the hydrogen donating ability of the antioxidant which leads to the formation of non-radical DPPH-H (Al-Farsi *et al*., 2005). The DPPH free radical scavenging activity of SLE was 76% (1mL) as compared to 88% in BHT (Fig.1). The strong free radical scavenging activity of SLE could be connected to the occurrence of a wide range of phenolic and polyphenolic compounds in SLE. The results of this investigation are corroborated with the earlier finding of Abbas *et al*. (2013) on the antioxidant activity of various sugarcane genotypes. The antioxidant and prophylactic activity of sugarcane has also been reported by Koge *et al*. (2000). Plant based natural antioxidants possess the capability of neutralizing free radicals, can safeguard the body from the damage caused to the cardiovascular system and oxidative stresses by the free radicals. DPPH free radical scavenging activity of sugarcane juice (60%) and detoxification of free radicals has been reported by Zamir *et al*. (2012). Li *et al*. (2010) studied the phenolics in sugarcane tip, stem, peel and leaves, the flavonoid content of the leaves 3700-µg/g was significantly higher over other parts of the plant ($P<0.05$).

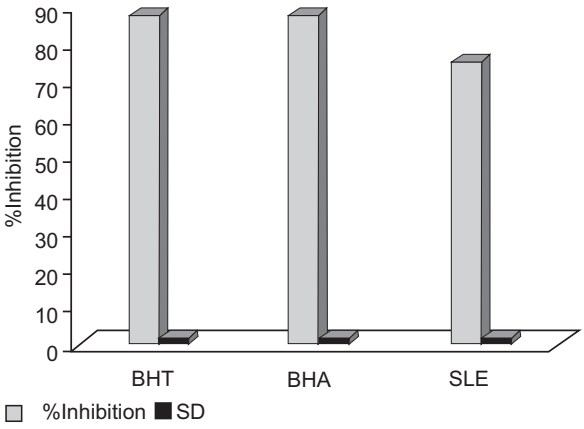

Fig.1. DPPH free radical scavenging activtiy of SLE. SD= standard deviation, BHT= butylated hydroxyl toluene; BHA= butylated hydroxyl anisole; SLE= sugarcane leaf extract.

Antioxidant activity in linoleic acid system. The antioxidant activity of SLE in linoleic peroxidation system is given in Fig. 2. SLE inhibited 82% peroxidation of linoleic acid as compared to 85% and 90% inhibition by BHA and butylated hydroxyl toluene (BHT) after 360 days of incubation. The percentage of inhibition of SLE was almost similar to BHA and comparable to (BHT). The great antioxidant activity of SLE can be connected to the higher concentration of phenolics. The

Table 1. Chemical composition of sunflower (SFO), soybean (SBO) and canola oils (CO)

Parameter	Unit	SFO	SBO	CO
Free fatty acids	%	0.08±0.01[a]	0.08±0.01[a]	0.09±0.02[a]
Colour	Lovibond scale	14±0.54[c]	18±0.92[b]	21±0.74[a]
Rancidity	Kries Test	-ve	-ve	-ve
USM	%	0.72±0.03[a]	0.66±0.04[a]	0.68±0.07[a]
Iodine value	Unit	122.46±1.29[a]	133.19±2.15[b]	110.54±1.42[c]

Within a row means denoted by a common letter are not statistically different; USM = Unsaponifiable matter.

Table 2. Effect of sugarcane leaf extract on peroxide value ($MeqO_2$/kg) of vegetable oils stored at ambient temperature

Treatments	0-Day	60-Days	120-Days	180-Days
SFO	0.25±0.03[n]	1.25±0.08[j]	2.88±0.11[d]	4.47±0.36[a]
SFO+SLE	0.25±0.03[n]	0.84±0.05[k]	1.34±0.07[i]	2.18±0.23[f]
SBO	0.22±0.02[n]	0.95±0.09[k]	2.53±0.15[e]	3.95±0.34[b]
SBO+SLE	0.22±0.02[n]	0.62±0.04[l]	1.74±0.13[h]	1.92±0.35[g]
CO	0.19±0.04[n]	0.81±0.05[k]	1.46±0.09[i]	3.19±0.46[c]
CO+SLE	0.19±0.04[n]	0.45±0.03[m]	1.22±0.07[j]	1.57±0.18

Within rows and columns means denoted by a different letter are statistically different (P<0.05).

antioxidant activity of *Moringa oleifera* leaf, fruits and vegetables for the inhibition of peroxidation of linoleic acid has been described by Anwar *et al*. (2006) and Siddhuraju and Becker (2003).

Peroxide value. The results regarding peroxide value of SFO, SBO and CO supplemented with SLE are given in Table 2. The addition of SLE in substrate oils considerably inhibited the generation of peroxides. Peroxide value of all the experimental samples and their controls increased throughout the storage period of 6 months but to varying extents. The rise of peroxide value during ambient storage period was dependent upon two factors, (i) the kind of oil i.e., degree of unsaturation and (ii) the addition of antioxidant. Polyphenolic compounds of SLE provided a great deal of protection to the oil samples added with SLE. Vegetable oils (controls) which were not added with SLE suffered a high degree of oxidation. The pattern of rise of peroxide value in the supplemented and non-supplemented vegetable oils was in the order of SFO> SBO > CO. The inhibition of lipid peroxidation phenomenon in the supplemented vegetable oils could be connected with the neutralization of free radicals by the antioxidant. The oxidizability of SFO was the highest followed by SBO and CO. The better inhibition of peroxidation could have been achieved by the application of higher dose of SLE however, this aspect needs to be further investigated. Assessment of peroxide value delivers a worthwhile evidence of the oxidative breakdown in fats and oils (Pritchard, 1991). Greater values are frequently interrelated with inferior storage stability (Fereidoon, 2005). The storage stability of vegetable oils through the phenolics of sugarcane leaf has not been previously investigated therefore, little is known regarding its suitability as an antioxidant for the preservation of vegetable oils at ambient temperature. The antioxidant activity of plant based natural antioxidants for the stabilization of vegetable and animal fat has been described by Nadeem *et al*. (2013); Anwar *et al*. (2011) and Mohdaly *et al*. (2011) .

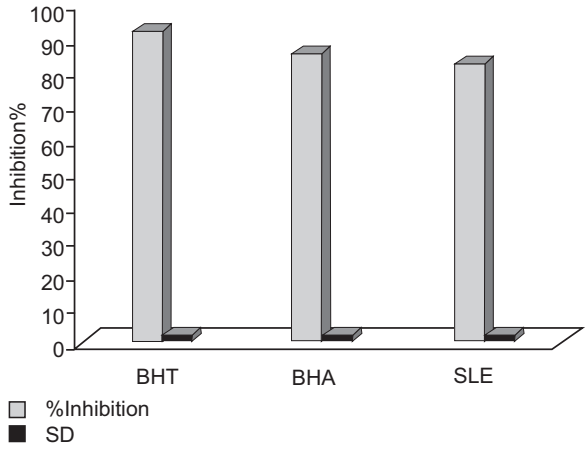

Fig. 2. Antioxidant activty of SLE in linoleic acid system. SD= standard deviation; BHT= butylated hydroxyl toluene; BHA= butylated hydroxyl anisole; SLE= sugarcane leaf extract.

Anisidine value. The results of anisidine value of vegetable oils added with SLE are presented in Table 3. Anisidine value of the experimental samples and their controls increased during the storage period of 180 days. Anisidine value of SFO supplemented with SLE and without SLE was highest at 60, 120 and 180 days of determination followed by SBO and CO and vegetable oils supplemented with SLE exhibited the lower concentration of oxidation products over their controls

(P<0.05). In this study, the effect of antioxidant on vegetable oils with different fatty acid composition was compared and the oxidizability of vegetable oils was in the order of SFO < SBO < CO. The generation of oxidation products also followed the fashion of oxidizability. The oxidation rate of linoleic acid is 12 times greater than oleic acid (Baer *et al*., 2001) that could be the justification of higher extents of oxidation products in SFO over SBO and CO. CO contains considerably higher concentration of monounsaturated fatty acids which are relatively more stable than polyunsaturated fatty acids that could be connected to the lower amount of oxidation products in CO. Determination of anisidine value gives information of the amount of oxidation products generated during the course of oxidative breakdown (Erickson, 1995). Addition of *Moringa oleifera* leaf extract considerably enhanced the storage stability of butter (Nadeem *et al*., 2013). The long term preservation of SFO and CO through barley and rice bran extracts has been reported earlier (Chatha *et al*., 2011; Anwar *et al*., 2010).

Table 3. Effect of sugarcane leaf extract on anisidine value of vegetable oils stored at ambient temperature

Treatments	0-Day	60-Days	120-Days	180-Days
SFO	4.11±0.04^{m}	14.64±0.62^{j}	23.72±1.13^{h}	47.96±1.54^{a}
SFO+SLE	4.11±0.04^{m}	9.62±0.47^{k}	17.68±0.91^{i}	34.67±1.18^{d}
SBO	4.31±0.02^{m}	12.95±0.77^{j}	32.54±1.64^{e}	41.18±1.76^{b}
SBO+SLE	4.31±0.02^{m}	8.29±0.59	13.67±2.16^{j}	29.22±0.86^{f}
CO	3.86±0.01^{m}	10.51±0.92^{k}	25.64±1.43^{g}	39.89±1.32^{c}
CO+SLE	3.86±0.01^{m}	7.62±0.38^{l}	12.38±0.66^{j}	19.35±0.43

Within rows and columns means denoted by a different letter are statistically different (P<0.05).

Conjugated dienes and trienes. The results of conjugated dienes and trienes of oils added with SLE and controls are given in Table 4 and 5. Oxidation products in the form of conjugated dienes and trienes went on increasing throughout the storage period of 180 days at ambient temperature depending upon the addition of natural antioxidant and fatty acid composition of individual oils. The rise of conjugated dienes and trienes at the end of storage period was in the order of SFO > SBO > CO, the determination frequencies of 60 and 120 days revealed the same order. In current investigation vegetable oils were compared with each other and their controls (without antioxidant). SFO, SBO and CO yielded the varying extents of oxidation product, also their extent was considerably lower than their controls (P<0.05) which revealed that the addition of SLE had a pronounced effect on the inhibition of auto-oxidation in these oils during long term storage at ambient temperature. The protective effect of SLE can be seen in all three types of 60, 120 and 180 days stored vegetable oils as compared to their controls. The inhibition of generation of oxidation products by SLE also has health beneficial effects as oxidation products have been implicated with flavour defects and health related disparities. Determination of conjugated dienes and trienes is regarded as one of the useful and convenient methods to quantify the oxidation products.

Table 4. Effect of sugarcane leaf extract on conjugated dienes of vegetable oils stored at ambient temperature

Treatments	0-Day	60-Days	120-Days	180-Days
SFO	0.21±0.030	3.65±0.17^{i}	8.92±0.31^{d}	19.87±0.49^{a}
SFO+SLE	0.21±0.030	1.18±0.11^{l}	3.57±0.21^{i}	7.84±0.33^{e}
SBO	0.15±0.020	2.98±0.24^{j}	7.51±0.17^{e}	16.84±0.71^{b}
SBO+SLE	0.15±0.020	1.02±0.14^{l}	2.94±0.16^{j}	5.63±0.25^{g}
CO	0.19±0.010	0.83±0.09^{m}	6.54±0.24^{f}	14.35±0.34^{c}
CO+SLE	0.19±0.010	0.55±0.07^{n}	2.56±0.12^{k}	4.29±0.10^{h}

Within rows and columns means denoted by a different letter are statistically different (P<0.05).

Table 5. Effect of sugarcane leaf extract on conjugated trienes of vegetable oils stored at ambient temperature

Treatments	0-Day	60-Days	120-Days	180-Days
SFO	0.08±0.021	1.85±0.08^{h}	4.53±0.29^{d}	8.76±0.41^{a}
SFO+SLE	0.08±0.021	1.79±0.05^{h}	2.19±0.18^{g}	3.41±0.10^{e}
SBO	0.08±0.021	1.35±0.13^{i}	3.43±0.14^{e}	7.64±0.22^{b}
SBO+SLE	0.08±0.021	0.59±0.04^{j}	1.42±0.08	2.76±0.16^{f}
CO	0.08±0.021	1.17±0.13^{i}	3.24±0.17^{e}	6.19±0.11^{c}
CO+SLE	0.08±0.021	0.42±0.05^{k}	1.22±0.04^{i}	2.14±0.07^{g}

Within rows and columns means denoted by a different letter are statistically different (P<0.05).

Changes in peroxide value in accelerated oxidation chamber. Schaal oven test is one of the simplest and widely used method to assess the antioxidant activity of antioxidants in the accelerated oxidation chamber at 63 °C for 5 days, the extent of oxidation was measured in terms of peroxide value. In the accelerated oxidation chamber SLE provided a high degree of protection to the supplemented oils (Fig. 3). Among the supplemented oils, the greatest peroxide value was exhibited by the

SFO followed by SBO and CO; similar trend was also seen in the non-supplemented vegetable oils. Enhancement of the oxidative stability of vegetable oils through the supplementation of SLE has not been previously investigated and this is the pioneer study suggesting the suitability of SLE for the long term stabilization of vegetable oils at room temperature. Fatty acid composition had a major effect on the rise of peroxide value in the oxidation chamber, oils rich in monounsaturated fatty acids are usually more stable to auto-oxidation, and therefore, CO yielded the lowest peroxide value as compared to SBO and SFO. Reduction in unsaturated fatty acids of SFO and SBO had a great effect on the enhancement of the oxidative stability of SBO and SFO (Anwar *et al*., 2007).

Induction period. Accelerated oxidation techniques are widely used for the assessment of antioxidant activity of antioxidants. Induction period of fats and oils is usually corroborated with keeping quality; higher values are usually connected with better storage stability and vice versa (Anwar *et al*., 2011). From the results of Fig. 4 it is obvious that SLE significantly improved the induction period of SFO, SBO and CO over their controls. In the absence of SLE, controls suffered from serious consequences of autoxidation during the long term storage and in the accelerated oxidation conditions (120 °C; 22 L air/h). The induction period of SLE supplemented vegetable oils were in the order of CO > SBO > SFO. The remarkable resistance of the supplemented vegetable oils in the accelerated oxidation conditions was due to the antioxidant activity of phenolics contained in SLE.

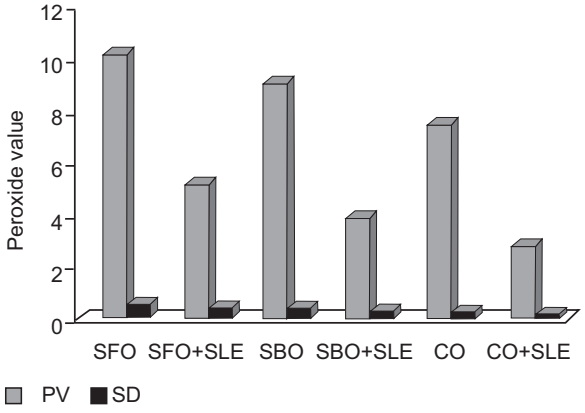

Fig. 3. Peroxide value (PV) of SLE added vegetable oils in Schaal oven test. SFO= sunflower oil; SLE = sugarcane leaf extract.

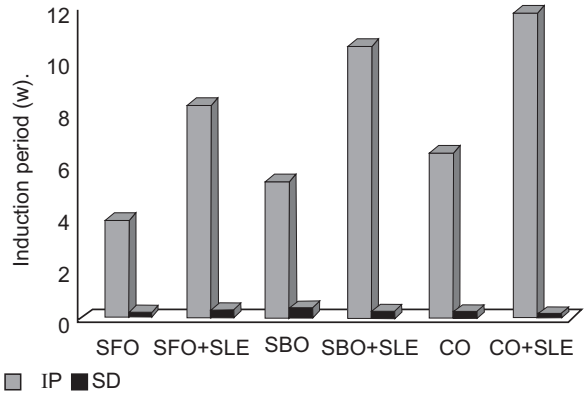

Fig. 4. Induction period (IP) of vegetable oils added with SLE. SFO = sunflower oil; SLE = sugarcane leaf extract.

Table 6. Effect of sugarcane leaf extract on fatty acid composition of sunflower, soybean and canola oils during storage period of 180-days at ambient temperature (mg/g)

Fatty acid	Sunflower oil			Soybean oil			Canola oil		
	Fresh	6M-SFO	6M-SFO+ SLE	Fresh	6M-SBO	6M-SBO+ SLE	Fresh	6M-CO	6M-CO+ SLE
C16:0	6.54±0.22e	10.38±0.39c	7.14±0.56d	10.41±0.21f	14.25±0.12a	12.55±0.12b	4.15±0.03f	7.32±0.09d	5.74±0.23df
C18:0	1.89±0.05e	3.15±0.27c	2.45±0.18d	3.55±0.95b	3.76±0.64b	2.38±0.09d	3.17±0.06c	4.45±0.08a	3.69±0.18b
C18:1	46.12±1.34d	42.59±0.95f	44.91±1.47e	22.95±2.14g	19.35±1.35i	21.13±1.31h	56.32±1.79a	53.67±2.16c	54.91±1.92b
C18:2	47.15±0.89c	40.29±1.38f	43.13±0.76e	51.19±0.12a	45.61±0.26d	48.97±0.23b	25.73±0.62g	22.47±1.13i	24.12±0.10h
C18:3	0.11±0.01f	0.03±0.01f	0.07±0.02f	6.19±0.64d	3.37±0.45e	5.67±0.14	10.35±0.49a	7.62±0.49c	9.24±0.17b

Within a row means denoted by a different letter are statistically different (P<0.05); Fresh=freshly deodorized vegetable oils; 6-M=six months stored oils at ambient temperature; 6M-SFO+SLE=sunflower oil supplemented with 600 ppm sugarcane leaf extract; 6M-SBO+SLE=Soybean oil supplemented with 600 ppm sugarcane leaf extract; 6M-CO+SLE=canola oil supplemented with 600 ppm sugarcane leaf extract.

Changes in fatty acid composition. The changes in fatty acid composition of vegetable oils as a function of addition of SLE and ambient storage are presented in Table 6. Major changes were observed in the fatty acid composition of controls, while, the change in fatty acid composition from the original value was not drastic in the SLE supplemented vegetable oils. Addition of SLE and degree of unsaturation were the two major factors responsible for the changes in fatty acid composition. The concentration of unsaturated fatty acids in 6 months stored controls and supplemented samples were less than the initial value due to their breakdown into oxidation products and increase in saturates was on percentage basis. C18:1 and C18:2 in fresh, 6 months stored controls and SLE supplemented SFO were 46.12%, 42.59%, 44.91% and 47.15%, 40.29, 43.13%, respectively. C18:2 and C18:3 in fresh and 180 days stored control and SLE supplemented SBO were 51.19%, 45.61%, 48.97% and 6.19%, 3.37,% 5.67%, respectively. C18:2 and C18:3 in fresh and 6 months stored CO were 25.73%, 22.47%, 24.12% and 10.35%, 7.62%, 9.24%, respectively. The phenolics of SLE provided a high degree of protection to the unsaturated fatty acids towards their breakdown into oxidation products which is evident from the lower generation of concentration of oxidation products in SLE supplemented vegetable oils. The strong correlation between degree of unsaturation and the generation of oxidation products during storage of oils rich in unsaturated fatty acids has been suggested by Gulla and Wagahary (2011). Addition of *Moringa oleifera* leaf extract in butter oil with higher extent of unsaturated fatty acids significantly inhibited the auto-oxidation phenomenon (Nadeem *et al*., 2013).

Conclusion

Supplementation of SFO, SBO and CO with sugarcane leaf extract at 600 ppm concentration significantly (<0.05) inhibited the auto-oxidation. Supplemented vegetable oils yield the lower extents of primary and secondary oxidation products with minimum changes in the fatty acid composition during storage period and improved resistance in the accelerated oxidation conditions, with no effect on viscosity and sensory characteristics. Sugarcane leaf extract therefore, can be used to prolong the shelf life of vegetable oils.

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Pak. j. sci. ind. res. Ser. B: biol. sci. 2016 **59**(3) 164-171

An Assessment of the Bivalve *Perna viridis*, as an Indicator of Heavy Metal Contamination in Paradise Point of Karachi, Pakistan

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(received September 3, 2015; revised May 6, 2016; accepted May 12, 2016)

Abstract. The edible bivalves *Perna viridis* (green mussel), (n = 100) were analysed for their total Hg, Pb, Cu, Ni, Zn, Co, Fe, Cr, Cd, and Mn concentrations to indicate heavy metal contamination in Paradise Point of Karachi coast using atomic absorption spectrophotometer. There are large seasonal variations in the metal concentrations of Mn (0.025-0.67 µg/g), Fe (0.055-7.740 µg/g), Ni (0.004-0.52 µg/g), Hg (0.0001-0.004 µg/g), Zn (0.04-3.32 µg/g), Cu (0.008-1.66 µg/g), Pb (0.022-2.43 µg/g), Co (0.01-0.044 µg/g), Cd (0.04-0.88 µg/g) and Cr (0.13-1.20 µg/g) recorded in bodies/soft tissues of *P. viridis* obtained in the samples of the year 1993 and 2012 at the Paradise Point of Karachi coast. The results of heavy metals are in the following descending order of concentration in the samples collected in the year 1993: Fe>Cr>Zn>Mn>Pb>Cd>Cu>Ni>Co>Hg, while Fe>Zn>Pb>Cu>Cr>Cd>Mn>Ni>Co>Hg order was recorded in samples collected in the year 2012. The high accumulation of metals was found mostly in the samples collected in the year 2012 when compared with the samples of the year 1993. This is an indication that the area under study showed signs of being exposed to significant levels of heavy metal pollution due to direct discharge of industrial and domestic wastes along the coast. The concentrations of these heavy metals were lower than the permissible limits for human consumption. However, if this pollution persists, it can prove to be very detrimental in future.

Keywords: Paradise Point, heavy metals, pollutants, industrial wastes, domestic wastes

Introduction

Marine pollution is a major threat to the health of millions of people, marine animals and plants. The pollution, especially of the metals (either heavy or trace) poses a direct threat to marine life and ultimately the human health (Qari and Siddiqui, 2004). The main sources of pollution in Karachi coastal water includes domestic and industrial waste, tanneries effluents, rainfall and associated pollutant from runoff, shipping and agri-cultural sources (Qari and Siddiqui, 2008). The rapid industrialisation and urbanisation of the city has altered the quality of the environment and created ecological disturbances and associated problems for the local community (Siddiqui *et al*., 2009).

Pollution in the Karachi coastal region is mainly attributed to the Lyari and Malir rivers, which are served by various channels of untreated domestic and industrial waste, carrying more than 300 million gallons per day (MGD) (1,125,000 m^3/day) of the untreated effluent of more than 6,000 industries. These rivers ultimately drain into the beaches of the Arabian sea (Hasnie and Qureshi, 2002; Rizvi *et al*., 1988). It is estimated that about 300 MGD of wastewater is generated in Karachi out of which only 40 MGD is treated, rest of the water waste and the treated effluents are discharged into the sea and creating harm to marine environment (Khattak *et al*., 2012). Thus, it has become important to estimate the heavy metal concentration in organisms which act as bio-accumulators in sediments, in overlying water, in fresh water, estuarine and marine environment because many of these organisms are indicator of metal contamination. Bio-monitoring by employing living organisms such as mussels, which is an economically important bivalve with outstanding potential as sentinel organism (Qari *et al*., 2015) and as sensor plays a vital role in governmental and industrial strategies to identify, assess, control, and reduce pollution problems (Krishnakumar *et al*. 1995; 1994). The intertidal portion of the exposed rocky shore at Paradise Point, the present study site, harbours a large population of *P. viridis*. The present

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investigation provides first-hand basic information about many aspects of bioecology of green mussel (*P. viridis*) from northern Arabian Sea. The aim of this present study is to determine the levels of contaminants in *P. viridis* from exposed shore of Paradise Point, Karachi coast at low tide and possible health risks to the consumers. The study also examines an increase of metal concentration levels with time in Paradise Point of Karachi coast.

Materials and Methods

Sampling. The green mussel, *P. viridis* (n = 100) were sampled from exposed shore of Paradise Point, Karachi coast (Fig. 1) at low tide in the year 1993 and 2012 for comparison of the data of two different periods. All the samples collected were carefully cleaned from mud debris and other epiphytes with filtered seawater, in the laboratory. Digestion of dried samples was carried out as described by Denton and Burdon-Jones (1986).

Sample preparation. *Perna viridis* samples were dried at 70 °C for 24 h till a constant weight was achieved. The samples were then homogenised with a porcelain pestle and mortar to a powder form, sieved and stored in plastic bottle until further analysis. Digestion of *P. viridis* samples was carried out as described by Denton and Burdon-Jones (1986). Triplicate samples of *P. viridis* (1 g) was digested with concentrated nitric acid (4 mL) and concentrated perchloric acid (2 mL) in 50 mL Teflon beaker (prewashed with nitric acid solution) covered with lid at 80 °C on a hot plate.

Analysis. After digestion and evaporation of acid, metal salts were re-dissolved in metal free deionised water and the final volume was made up to 100 mL in volumetric flask. Standards were prepared in deionized water from stock standard AA solution (May and Baker Ltd., Dagenham, England). Reagents blank were treated similarly as samples using same volume of acid and deionised water. In digested samples of *P. viridis*, concentrations of Hg, Pb, Cu, Ni, Zn, Co, Fe, Cr, Cd and Mn were measured by Atomic Absorption Spectrophotometer.

Results and Discussion

There are large seasonal variations in metal concentrations of Mn (0.025-0.67 µg/g), Fe (0.055-7.740 µg/g), Ni (0.004-0.52 µg/g), Hg (0.0001-0.004 µg/g), Zn (0.04-3.32 µg/g), Cu (0.008-1.66 µg/g), Pb (0.022-2.43 µg/g), Co (0.01-0.044 µg/g), Cd (0.04-0.88 µg/g) and Cr (0.13-1.20 µg/g) recorded in bodies/soft tissues of *P. viridis* collected as samples in the year 1993 and 2012 at the Paradise Point of Karachi coast (Fig. 2-11). The values of Fe, Zn, Cu, Cd and Pb concentrations in *P. viridis* collected from the exposed shore of Paradise Point of Karachi coast were compared to permissible limits from established guidelines for food safety (FDA, 2001;

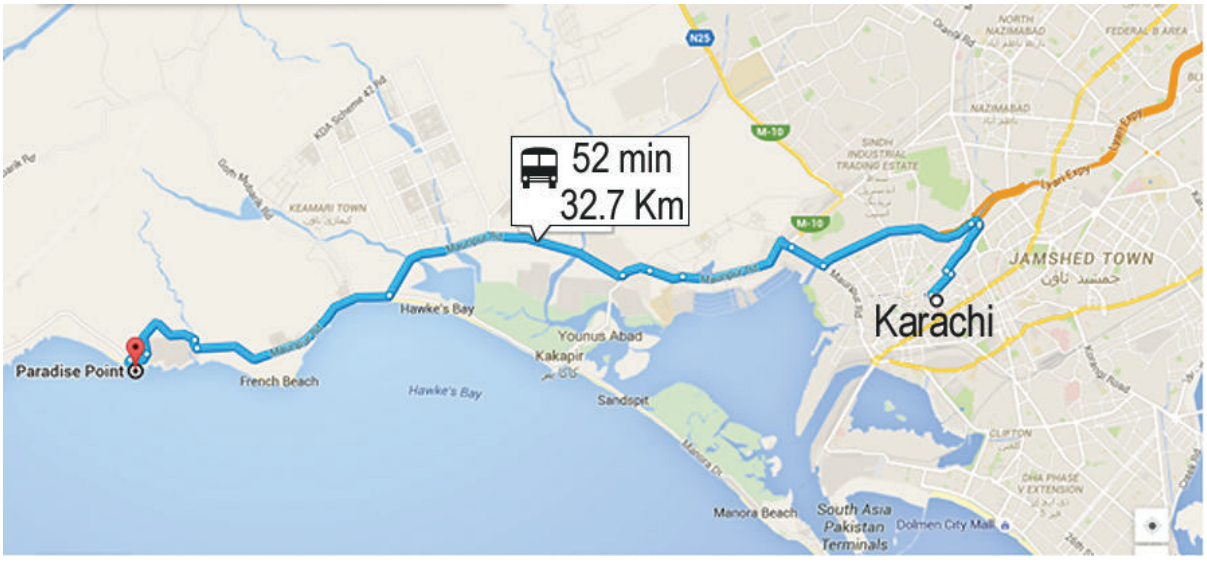

Fig. 1. Map of the Karachi coast showing location of the sampling beach (Paradise Point), source: www.google.com

WHO, 1982) and maximum permissible limits established by Malaysian Food Regulations (MFR, 1985) (Table 1). The observed concentrations of these metals were within the maximum permissible level (MPL) as shown in Table 1. However, if this pollution persists, it can prove to be very detrimental in future.

All the studied metal levels (Hg, Pb, Cu, Ni, Zn, Co, Fe, Cr, Cd, and Mn) found in the green mussels, *P. viridis* collected from exposed shore of Paradise Point, Karachi coast at low tide in the year 2012 were higher than those detected in mussels collected in 1993. The results are shown in (Fig. 2-11). This is an indication that the area under study showed signs of being exposed to significant levels of heavy metal pollution due to direct discharge of industrial and domestic wastes along the coast. The results of heavy metals concentrations in the following descending order in the samples collected in the year 1993 were Fe>Cr>Zn>Mn>Pb>Cd>Cu>Ni>Co>Hg, while Fe>Zn>Pb>Cu>Cr>Cd>Mn>Ni>Co>Hg were recorded in samples collected in the year 2012. The results of the present study have therefore, confirmed that the green mussels (*P. viridis*) have greater capacity for accumulation of metals.

The large seasonal variation recorded in the metal concentrations from the exposed shore of Paradise Point, Karachi coast in the year 1993 and 2012 could be as a result of upwelling on the composition of coastal water. High concentrations of metals also indicate anthropogenic influences (Qari and Siddiqui, 2004). The present ranges of concentration of metals (Hg, Pb, Cu, Ni, Zn, Co, Fe, Cr, Cd, and Mn) were higher when compared with the baseline composition of seawater (Riley and Skirrow, 1975; Horne, 1969). This could be due to the direct discharge of industrial and domestic wastes (along the coast) by Lyari river and the riverborne trace metals. The erosion of the shores at the bottom, diffusion from shelf sediments and deposition of atmospheric particulate also increased the metal concentration levels. Another source of pollution is Karachi atomic nuclear power plant located near the Paradise Point. This heavy water reactor has a generating capacity of 137 megawatt. It uses 0.15 million gallons of seawater per minute for cooling purpose. Chlorine is used for the control of bio-fouling problems in this plant, which discharge stack of gases in the atmosphere and liquid wastes, radioactive substances, a number of heavy metals and heated water sub-tidally on the nearby rock shore.

The trends of accumulation of heavy metals were also worked out with respect to seasons/months at low tide in the year of 1993 and 2012 for comparison of the data of two different periods. For the present probe, the metal variations are recorded in two seasons i.e., summer (starting from April-September) and winter (from October-March). The study showed that the concentration levels of most of the metals recorded in the soft tissues of *P. viridis* varied from season to season, year to year and even month to month. The highest concentration of Mn (0.67 µg/g) was measured in the month of October 2012 and lowest (0.025 µg/g) in the month of May 1993 as shown in (Fig. 2). The maximum concentration of Fe (7.740 µg/g) was measured in August 2012 and minimum level (0.055 µg/g) in July 1993 as shown in (Fig. 3). The highest Ni concentration (0.52 µg/g) was recorded in August 2012 while the least (0.004 µg/g) was recorded in July 1993 as shown in (Fig. 4). The maximum concentration of Hg (0.004 µg/g) was measured in February 2012 and the minimum level (0.0001 µg/g) in October 1993 as shown in (Fig. 5). The highest concentration of Zn (3.32 µg/g) was measured in June 2012 while the least concentration (0.04 µg/g) were observed in May 1993 as shown in (Fig. 6). The maximum concentration of Cu (1.66 µg/g) was recorded in November 2012 and

Table 1. Comparison of different heavy metals (Fe, Zn, Cu, Cd and Pb) concentrations in soft tissue of *P. viridis* sampled from Paradise Point of Karachi with other sampling locations from previous studies and International regulation value for food

Sampling area	Cu	Cd	Zn	Pb	Fe
			(µg/g)		
Present study (Paradise Point, Karachi, Pakistan)	0.008-1.66	0.04-0.88	0.04-3.32	0.022-2.43	0.055-7.740
Certified reference material (CRM)	2.34±0.16	0.043±0.008	25.6±2.3	0.065±0.007	142±10
Maximum permissible levels (MPL) (WHO, 1982)	10	2	100	5	-
Maximum permissible levels (MPL) (FDA, 2001)	100	0.2	150	1.5	-
Maximum permissible levels (MPL) (MFR, 1985)	30	1	100	2	-

the least (0.008 µg/g) in May-July 1993 as shown in (Fig. 7). The highest Pb (2.43 µg/g) concentration was detected in September 2012 and the lowest (0.022 µg/g) in May 1993 as shown in (Fig. 8). Co and Cd had the highest levels (0.044 µg/g, 0.88 µg/g) in May and July 2012, respectively, and lowest (0.01 mg/L, 0.04 mg/L) in January1993 as shown in (Fig. 9) and (Fig. 10). The highest mean of Cr (1.20 µg/g) was detected in February 2012 and the lowest (0.13 µg/g) in February 1993 as shown in (Fig. 11). Mostly, the high concentration of metals was found in summer and low in winter (Figs. 2-11).

Higher concentrations of heavy metals like Fe, Pb, Zn, and Cu in the green mussel (*P. viridis*) had been reported by Qari *et al*. (2015); Ali *et al*. (2014); Sasikumar *et al*. (2006); Krishnakumar *et al*. (1998) and Sankaranarayanan *et al*. (1976), while Fe accumulation was highest among the metals studied. Similarly, higher concentrations of heavy metals like Pb, Cd, Cr and Cu were also observed in *P. viridis* as reported by Blackmore and Wang (2003) and Shin *et al*. (2002). The port and several industries are located in the Karachi city. Untreated or partially treated industrial and domestic waste waters which are discharged directly or carried by several streams into the Karachi coastal water could be attributed to the higher concentrations of heavy metals such as Fe, Zn, Pb, Cd, Cr, Cu and other heavy metals recorded in the soft tissues of *P. viridis* in Paradise Point of Karachi. Studies conducted by Qari and Siddiqui (2004) showed that concentrations of Mn, Cu, Zn, Cr and Pb and other metals in coastal areas are believed to be elevated due to discharge of considerable quantities of untreated and partially treated domestic and industrial wastes.

The concentration of Fe was the highest as compared to other heavy metals at low tides in both years i.e., 1993 and 2012 (Fig. 3). It is assumed that high input of metals and other inorganic and organic substances are coming in the form of industrial and domestic wastes in Paradise Point, Karachi coast. These anthropogenic sources play a major role in increasing the concentration of Fe in Paradise Point, Karachi coast. The main sources of Fe are mostly from the industries situated in the coastal area. The pollutants from the industries are directly poured into the stream from where it has been mixed with the sea water. The low residence time in oxidizing environment favours the rapid precipitations of iron (Khattak *et al*., 2012). The observed highest concentration of Fe in the soft tissue of *P. viridis* in the two periods (1993 and 2012) when compared to other heavy metals detected in the soft tissues of the green mussels was clearly indicating the tendency of organism in accumulating higher concentration of Fe in soft tissue. This might also be due to the major role played by this essential metal in catalysing various enzymatic activities (Kamaruzzaman *et al*., 2011).

High concentrations of Cu, Zn, Cr, Pb and other metals detected in *P. viridis* sampled at the exposed shore of Paradise Point, Karachi coast in the two periods (1993 and 2012) are also due to harbour activities such as dredging and cargo handling, the dumping of ship waste and other coastal activities (Qari and Siddiqui, 2008). It was also reported by Ansari *et al*. (2001) that the concentration of Cu, Ni, Zn, Pb, Cr and Cd were high in sludge samples of industrial area. Considering these facts, the present study asserted the research findings by those authors (Ansari *et al*., 2001). According to Qari and Siddiqui (2008), coastal areas with heavy metal pollution needs special attention because continuous pollution inventories will have adverse effect in terms of increase in toxicity levels of the marine food chain, stress on marine plants and animals, adverse effect on the health of inhabitants such as fisher men and bathing tourist. Urgent attention is needed to combat pollution in marine environment of Karachi coast.

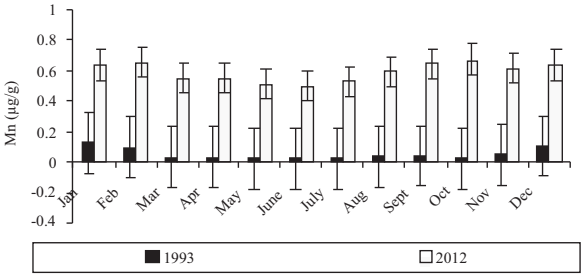

Fig. 2. Mn concentration in *P. viridus* in two different periods.

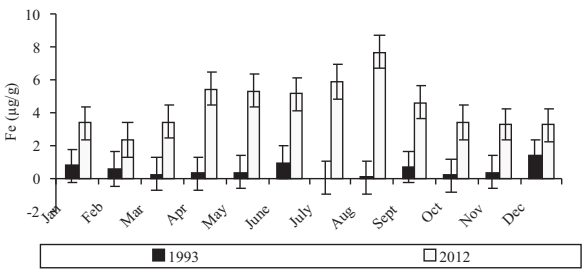

Fig. 3. Fe concentration in *P. viridus* in two different periods.

The highest concentration in cadmium (Cd) causes several health problems in human. Cadmium and its compounds along with mercury and some other dangerous metals are, however, included in the blacklist. It is being used routinely in different industrial processes and its potential hazard to life form is predominant. Eating food or drinking water with very high cadmium levels severely irritates the stomach, leading to vomiting and diarrhoea, and sometimes death. Eating lower levels of cadmium over a long period of time can lead to a build-up of cadmium in the kidneys. If it reaches a high enough level in the kidney it will cause kidney damage, and also causes bones to become fragile and break easily. Cadmium (Cd) is widely distributed at low level in the environment and most foods have an inherently low level of Cd which has been shown to bind to the protein and accumulate significantly in higher level (FDA, 2001). Ololade *et al*. (2008) reported that Cd level is almost 10 times higher in shell fishes than in fin fishes. The transport of fertilizer in nearby areas to the estuaries by leaching and erosion as a result of agricultural activities apart from fishing by the village folks could be responsible for the higher level of Cd in shell fishes than in fin fishes. On the other hand, it has been observed that bivalves do not regulate Cd in their body tissues. According to Li *et al*. (2006), bivalves do not regulate Cd therefore, accumulate this element in their body. In view of this reason, it is plausible that bivalves such as *P. viridis* might not be able to regulate Cd in their body.

Mercury (Hg) is one of the heavy metals in the marine ecosystem that are of great concern if present at an elevated level as it could have hazardous impacts due to its toxicity (Yap *et al*., 2007). Evidence of this concern to public health was given by other researchers reporting on Hg contamination in the biota related to human beings (Zhou and Wong, 2000; Moraes *et al*., 1997; Leah *et al*., 1982). However, the concentration of Hg was the lowest as compared to other heavy metals analysed in present investigation in the two periods (1993 and 2012). The low concentrations of Hg detected in the soft tissues of *P. viridis* in the present study, could be due to the fact that metallothionein might play a role in regulating the Hg excretion as well as storage (Roesijadi, 1980). The lowest Hg levels observed in the present study could also be due to the metal-binding proteins being present at much lower concentrations in *P. viridis* body tissues. The observed low concentration of Hg in the soft tissue of the organism was clearly

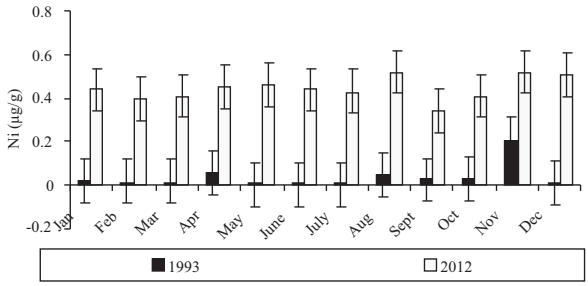

Fig. 4. Ni concentration in *P. viridus* in two different periods.

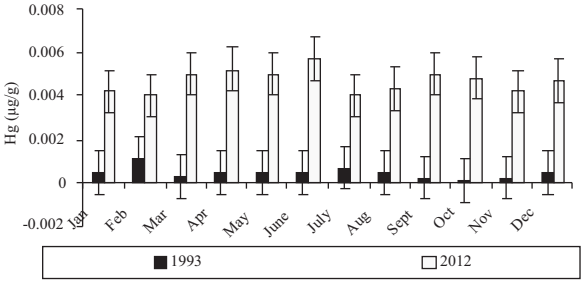

Fig. 5. Hg concentration in *P. viridus* in two different periods.

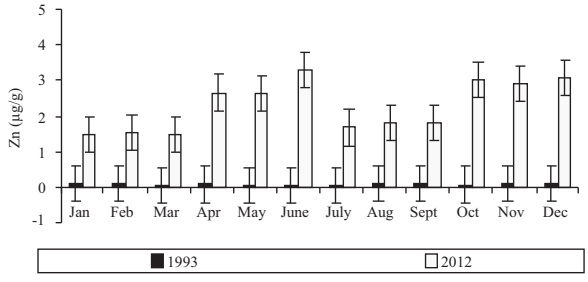

Fig. 6. Zn concentration in *P. viridus* in two different periods.

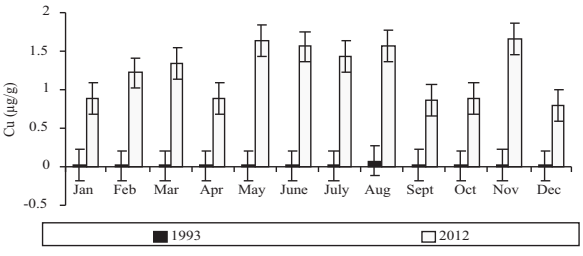

Fig. 7. Cu concentration in *P. viridus* in two different periods.

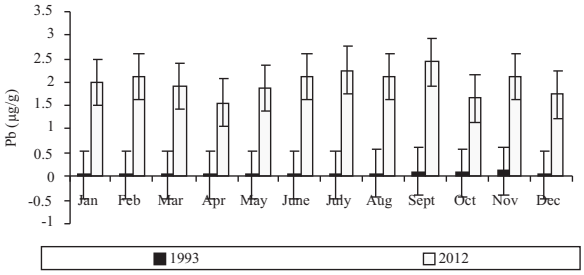

Fig. 8. Pb concentration in *P. viridus* in two different periods.

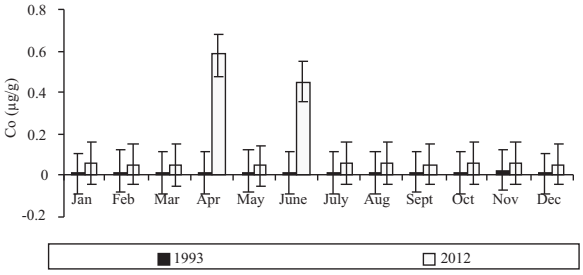

Fig. 9. Co concentration in *P. viridus* in two different periods.

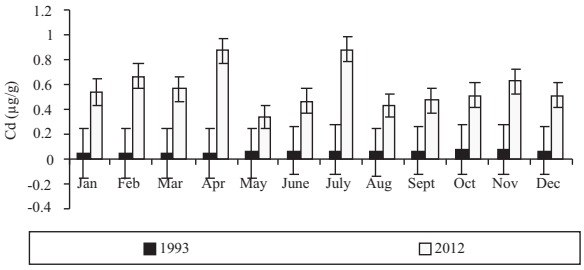

Fig. 10. Cd concentration in *P. viridus* in two different periods.

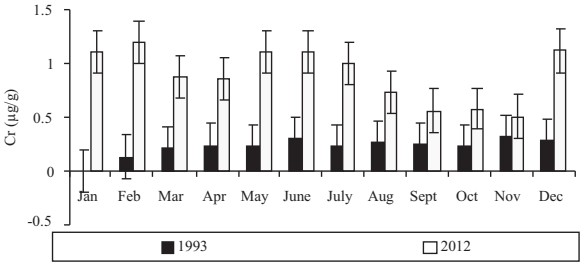

Fig. 11. Cr concentration in *P. viridus* in two different periods.

indicating the tendency of organism in accumulating lower concentration of Hg in soft tissue. In a study conducted by Usero *et al*. (2005), Hg showed the lowest values among all the metals studied on heavy metal concentrations in molluscs from the Atlantic coast of southern Spain.

The data generated from this study, showed that the concentrations of metals in the mussels collected from the Paradise Point are increasing with time. Obviously, the present investigation recorded highest concentrations of heavy metals in the year 2012 when compared to the year 1993. On a normal basis, it was expected that the levels of the heavy metals in 2012 would have drop drastically after the first detection levels of the heavy metals in the year 1993 but the reverse was the case. This is an indication that the Paradise Point of the Karachi coastal areas is subjected to intense industrial and anthropogenic activities and that coastal population encroachment and sewerage system plays an important role in the increase of metal concentration levels. Besides, there is an accelerating accumulation of toxic metals and gases in atmosphere, irrigation water and agricultural soils while the industrial estates of Karachi are discharging large quantities of effluents of organic matter, heavy metals, oil, greases, liquid and solid wastes into Malir and Lyari rivers, which are causing serious environmental degradation (Khattak *et al*., 2012) to various ecosystems of the city.

Conclusion

The concentrations of these heavy metals were within the maximum permissible level (MPL) and should result in no acute toxicities of the metals since they were lower than the permissible limits for human consumption. In addition, these metal concentrations were also considered to be low when compared with regional data based on *P. viridis* as a biomonitoring agent. However, the data generated from this study, showed that the concentrations of metals in the mussels collected from the Paradise Point are increasing with time. Obviously, the higher accumulations of metals were found mostly in the samples collected at the Paradise Point of Karachi coast in the year 2012 when compared with the samples collected in the year 1993 at the same sampled site. This is an indication that the area under study showed signs of being exposed to significant levels of heavy metal pollution due to direct discharge of industrial and domestic wastes along the coast. In view of this, continued monitoring of heavy metals from Paradise Point of Karachi coast should be undertaken to avoid reduction in the valuable export of bivalves and shrimps as well as the reduction of marine life in the coastal waters.

The most important large industries in Karachi involve metal and non-metal manufacturing textiles, tobacco, food and beverages, chemicals, paints, rubber, paper and paper product, pharmaceutical and product of coals and oil that contributes approximately 99% of the total industrial pollution (Khan and Saleem, 1988; Haq, 1976) which resulted to a considerable trace metal contamination of the marine environment. Immediate measures to control the indiscriminate discharge of effluent and domestic sewage directly or indirectly in to the sea need to be addressed. In addition, regular monitoring programmes of marine pollution are essentially required and need to be well implemented. If this pollution persists, it can prove to be very detrimental in future. The use of green mussel (*P. viridis*) as a suitable biomonitor for heavy metals concentration could be employ in programmes designed to identify, assess, control, and reduce pollution problems in the coast of Paradise Point of Karachi.

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Pak. j. sci. ind. res. Ser. B: biol. sci. 2016 **59**(3) 172-174

Short Communication

Early Growth Behaviour of Wheat Genotypes as Affected by Polyethylene Glycol (PEG-6000)

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(received August 27, 2014; revised April 22, 2016; accepted May 9, 2016)

Abstract. Polyethylene glycol (PEG-6000) is often used for the early establishment screening of the wheat genotypes against drought stress conditions. A collection of twenty-one newly developed bread wheat genotypes (developed through conventional and mutational breeding techniques) and four commercial drought tolerant check varieties were included in screening at seedling stage under three treatments of PEG-6000; T1 (0.5 MPa) and T2 (0.75 MPa) along with control T3 (distill water only). Three important early growth establishment traits like germination percentage, root length and shoot length of the wheat genotypes were observed. Significant variation among genotypic means regarding the observed traits was recorded at both the treatments. Wheat genotypes performance including check varieties for germination percentages was recorded as T1 (86.1%), T2 (72.4%) with reduction percentage of 12.5% and 33.8%. Root length of genotypes decreased by 37.0% in 0.5 MPa and 82.75% in 0.75 MPa, whereas, shoot length reduction was recorded as 38.9% (T1) and 84.6% (T2) as compared to control. This study provided essential information about the performance of advanced wheat genotypes under water stress conditions at early seedling establishment.

Keywords: moisture stress, PEG-6000, germination percentage, root length, shoot length

Wheat (*Triticum aestivum* L.) is staple food source and leading cereal crop of Pakistan (Anwar *et al*., 2011). It adds 10.0% value in agriculture and 2.1% to the GDP with the yield of 25.478 million tonnes (PESR, 2015). Moisture stress is one of the environmental constraints of wheat along with salinity and high temperature (Sial *et al*., 2013; 2007; Blum *et al*., 1980), which reduces plant growth, development and ultimately causes reduction in the productivity. Early seedling establishment is a very critical growth stage to determine the tolerance of genotypes to moisture stress (Baloch *et al*., 2012). Water stress during early stages can adversely affect plant growth and ultimately its final yield (Sial *et al*., 2013). Tolerance to biotic and abiotic stresses and ultimate high potential for grain yield determines the productivity of the wheat genotypes (Seher *et al*., 2015).

Several drought-related morphological and physiological characteristics under *in vivo* field conditions have been proved to be useful in screening of drought studies in wheat (Razzaq *et al*., 2013). The early seedling water stress can be evaluated *in vitro* through different techniques by artificially imposing drought stress through high molecular weight chemicals such as polyethylene glycol (PEG) (Khan *et al*., 2012). Earlier studies reported that PEG can induce stress to the plant in a relatively controlled manner through modifying the osmotic potential of the nutrient solution culture (Meneses *et al*., 2011; Zhu *et al*., 1997).

Present studies were designated to observe the effects of artificial osmotic stress at early seedling stage of wheat genotypes induced through two PEG-6000 concentrations. 21 newly evolved wheat genotypes (advance/mutant lines) along with four check commercial wheat varieties were screened at Nuclear Institute of Agriculture (NIA), Tandojam Sindh, Pakistan. Moisture stress was imposed through two levels of PEG-6000 (T1=0.5 MPa, T2=0.75 MPa) along with control (T3=0.0 MPa). The experiment was conducted in complete randomized design (CRD) with 3 replications. Healthy seeds of each wheat genotype were surface sterilized with 5% sodium hypochlorite (NaOCl) solution for 10 min, followed by washing with distilled water for several times on a blotting paper. Surface sterilized 10 seeds of each genotype were sown separately for germination on soaked filter paper using sterilized petri dishes. Filter papers in the petri dishes of T1 and T2 were moisturized by adding 10 mL PEG-6000 solution with 0.5 MPa and

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0.75 MPa concentrations, respectively, whereas normal sowing with distilled water was applied in T3 as control. Data pertaining to the germination percentage, root length (cm) and shoot length (cm) were recorded after eight days of sowing from randomly selected seedlings. Data were statistically analysed for analysis of variance (ANOVA) and means were compared using Duncan's multiple range test (DMRT). Percent reduction in root and shoot length, root length drought tolerance index and shoot length drought tolerance index of each wheat genotype was calculated.

Significant variability among the means of the wheat genotypes for germination percentage, root length and shoot length was observed in both T1 (0.5 MPa) and T2 (0.75 MPa) treatments as induced by PEG-6000 with comparison to control T3 (distill water) (Table 1). Overall performance of all wheat genotypes including check varieties for germination percentages was recorded as 86.1% in 0.50 MPa and 72.4% in 0.75 MPa with the respective reduction percentages of 12.5% and 33.8%. Highly significant and the highest means for germination percentage under T1 (0.5 MPa) and T2 (0.75 MPa) was found among the NIA-8/7 (T1=94.6%; T2=83.6%), NIA-10/8 (T1= 93.6%; T2=81.6%), SI-9196 (T1=93.0%; T2=81.6) which suggested that these genotypes could be drought-tolerant and possess early growth vigour during early seedling stage. Wheat genotypes NIA-28/4 and BWM-84 both had lowest germination percentages with the respective values of 73.3% and 73.0% under T1 (0.5 MPa), while highly significant and lowest means for germination percentage under T2 (0.75 MPa) was expressed by NIA-30/5 (61.6%), SI-9590 (61.0%), MSH-17 (60.6%), BMW-84 (62.3%) and BWM-47 (61.0%). Root length reduction percentage of all the genotypes at T1 and T2 was calculated as 37.0% and 82.7%, respectively, as compared to T3. *In vitro* development of root length at seedling stage provides a valid estimate about the root growth in field conditions (Bibi *et al*., 2012). Wheat genotype SI-9196, followed by ESW-9525 showed significantly maximum root length with the values of 14.07 cm and 13.63cm. NIA-25/1 had the maximum (95.6%) root length reduction percentage at T1 as compared to the control. Shoot length of wheat genotypes showed the overall reduction in means by

Table 1. Effect of f PEG-6000 on germination percentage, root length and shoot length of wheat genotypes

Genotypes	Germination (%)			Root length (cm)			Shoot length (cm)		
	T1	T2	T3	T1	T2	T3	T1	T2	T3
BWM-3	86.6^{abc}	76.0^{abc}	95.0^{ab}	10.8^{cde}	8.36^{ef}	13.9^{bcd}	17.7^{abc}	13.1^{bcd}	21.5^{abc}
NIA-8/7	94.6^{a}	83.6^{a}	100.0^{a}	12.4^{abc}	9.80^{abc}	15.4^{abc}	19.6^{a}	15.5^{a}	22.9^{abc}
NIA-9/5	91.3^{ab}	74.3^{abc}	96.6^{ab}	9.73^{bcd}	7.63^{j}	10.8^{e}	12.3^{jkl}	11.7^{def}	19.7^{bcd}
NIA-37/6	89.6^{ab}	62.3^{f}	93.3^{bc}	11.5^{bcd}	9.46^{bcd}	15.1^{abc}	12.9^{h}	11.2^{efg}	20.6^{bcd}
NIA-10/8	93.6^{a}	81.6^{a}	96.6^{ab}	11.9^{abc}	8.53^{def}	17.1^{ab}	12.4^{jkl}	13.8^{abc}	19.1^{cde}
NIA-25/1	87.6^{ab}	67.3^{cde}	100.0^{a}	8.96^{hij}	5.83^{g}	17.6^{ab}	14.2^{fgh}	11.2^{efg}	20.8^{bcd}
NIA-28/4	73.3^{e}	63.3^{ef}	86.6^{d}	9.23^{gh}	6.06^{g}	11.0^{e}	15.6^{cde}	8.63^{h}	22.7^{abc}
NIA-30/5	77.0^{de}	61.6^{f}	90.0^{cd}	9.16^{ghi}	7.13^{fg}	14.3^{abc}	13.1^{gh}	8.96^{gh}	14.7^{i}
NIA-25/5	80.0^{cde}	78.3^{abc}	93.3^{bc}	8.9^{hij}	6.46^{g}	11.0^{e}	15.6^{cde}	10.3^{fgh}	22.7^{abc}
ESW-9525	91.0^{ab}	83.0^{a}	99.3^{a}	13.6^{ab}	9.30^{bcd}	16.7^{ab}	19.1^{ab}	16.2^{a}	22.9^{ab}
SI-9196	93.0^{a}	81.6^{a}	99.3^{a}	14.0^{a}	11.47^{a}	16.5^{ab}	18.3^{abc}	15.0^{ab}	21.8^{abc}
SI-9590	86.6^{abc}	61.0^{f}	93.0^{bc}	10.1^{efg}	7.10^{fg}	12.6^{cde}	11.2^{l}	9.96^{fgh}	20.2^{bcd}
MSH-14	90.0^{ab}	79.6^{ab}	98.0^{ab}	12.2^{abc}	9.36^{bcd}	16.3^{abc}	16.7^{bcd}	12.4^{cde}	22.6^{abc}
MSH-17	76.6^{de}	60.6^{f}	95.0^{ab}	9.06^{ghi}	6.00^{g}	11.6^{de}	12.6^{ijk}	9.16^{gh}	20.4^{bcd}
MSH-36	88.6^{ab}	79.0^{ab}	99.3^{a}	11.2^{cde}	8.63^{cde}	14.0^{bcd}	15.4^{cde}	12.3^{cde}	18.4^{def}
MSH-22	83.3^{bcd}	66.6^{def}	99.3^{a}	11.1^{cde}	8.70^{bcd}	15.4^{abc}	10.8^{l}	9.96^{fgh}	20.4^{bcd}
BWQ-4	87.3^{abc}	68.6^{bcd}	100.0^{a}	13.5^{ab}	10.2^{abc}	17.4^{ab}	17.5^{abc}	11.1^{efg}	20.0^{bcd}
BWS-77	76.6^{de}	63.3^{ef}	99.6^{a}	8.63^{ij}	6.36^{g}	14.5^{abc}	16.4^{bcd}	9.13^{gh}	24.6^{a}
BWM-84	73.0^{e}	62.3^{f}	95.3^{ab}	9.50^{fgh}	6.33^{g}	11.7^{de}	12.2^{kl}	8.90^{gh}	16.8^{hi}
BWS-78	89.6^{ab}	80.6^{a}	100.0^{a}	13.0^{abc}	10.3^{abc}	18.1^{a}	18.3^{abc}	10.7^{efg}	21.1^{abc}
BWM-47	77.0^{de}	61.0^{f}	96.0^{ab}	9.33^{fgh}	5.66^{g}	15.5^{abc}	12.3^{jkl}	7.6 0^{l}	17.5^{fgh}
Sarsabz (Check)	92.0^{a}	82.6^{a}	99.6^{a}	12.1^{abc}	9.30^{bcd}	16.6^{ab}	15.1^{def}	11.8^{def}	21.8^{abc}
Thorhi (Check)	91.6^{a}	77.0^{abc}	98.6^{a}	10.7^{def}	10.4^{ab}	16.8^{ab}	14.7^{efg}	12.0^{cde}	18.0^{efg}
Margalla-99 (Check)	92.0^{a}	74.3^{abc}	99.6^{a}	10.0^{efg}	10.2^{abc}	14.9^{abcd}	12.3^{jkl}	10.9^{efg}	20.4^{bcd}
Chakwal-86 (Check)	90.3^{ab}	80.0^{ab}	99.0^{a}	12.4^{abc}	10.2^{abc}	16.5^{ab}	15.8^{cde}	14.2^{abc}	17.3^{gh}
Mean	86.1	72.4	96.9	10.9	8.4	14.8	14.9	11.4	20.4

T1 = (0.5 MPa); T2 = (0.75 MPa); T3 = control (0.0 MPa); values followed by the common letters are not significant to each other at $p<0.05$.

applying PEG-6000 concentrations as 14.9 cm (T1=0.5 Mpa), 11.4 cm (T2=0.75 MPa) and 20.4 cm (T3=0.0 Mpa) (Table 1). Reduction percentage of the shoot length on an average was found as 38.9% (T1) and 84.6% (T2). Wheat genotypes NIA-8/7 (19.60 cm) and ESW-9525 (19.10 cm) expressed the highly significant and the maximum values for shoot length under T1 (0.5 MPa) than all other genotypes. These results suggested that the genotypes NIA-8/7 and ESW-9525 could be more tolerant to moisture stress imposed at early growth stage. Present studies have provided the valid information regarding stress tolerance in newly evolved genotypes. The promising wheat genotypes NIA-8/7 and ESW-9525 along with MSH-22, NIA-9/5, NIA-10/8 and SI-9196 will be further evaluated in future breeding for improvement of water stress tolerance.

Table 2. Decrease (%) in germination percentage, root length and shoot length of wheat genotypes due to PEG-6000 induced stresses (T1, T2) as compared to control (T3)

Genotypes	Germination (%)		Root length (cm)		Shoot length (cm)	
	T1	T2	T1	T2	T1	T2
BWM-3	9.6	25.0	29.6	66.7	22	64.9
NIA-8/7	5.6	19.5	24.2	57.1	16.8	47.7
NIA-9/5	5.9	30.1	12.4	43.4	58.9	68.4
NIA-37/6	4.0	49.8	31.3	58.9	60.5	84.8
NIA-10/8	3.2	18.4	44.5	102.4	54	37.4
NIA-25/1	14.0	48.6	95.6	203.4	46.2	86.6
NIA-28/4	18.3	37.0	19.6	80.3	46.2	165.1
NIA-30/5	16.9	45.9	55.4	101.4	11.4	63.3
NIA-25/5	16.6	19.2	24.7	70.8	45.2	119.2
ESW-9525	9.1	19.6	22.8	79.6	20.4	42
SI-91196	6.8	21.5	17	43.5	19.7	45
SI-9590	7.3	52.5	23.5	77.5	81.3	103
MSH-14	8.9	23.0	32	71.3	35.3	80.8
MSH-17	23.9	56.5	28.6	95	61.4	122.8
MSH-36	12.0	25.7	23.9	62.8	18.7	49.6
MSH-22	19.2	48.9	38.7	77	89.8	105
BWQ-4	14.5	45.6	29.6	71.6	14.3	80.2
BWS-77	30.0	57.5	68.6	126.6	50.6	171.4
BWM-84	30.5	53.0	23.2	85.7	37.7	88.8
BWS-78	11.5	23.9	39.2	74	15.3	97.2
BWM-47	24.7	57.4	67.7	173.7	43.1	131.6
Sarsabz	8.4	20.6	36.1	78.5	43.4	84.7
Thori	7.6	28.2	56.5	61	23.1	50.8
Margalla-99	8.4	34.2	49	44.7	64.5	87.2
Chakwal-86	9.6	23.8	32	61.8	9.5	21
Mean	12.5	33.8	37	82.75	60.5	84.8

T1= (0.5 MPa); T2= (0.75 MPa); T3=Control (0.0 MPa).

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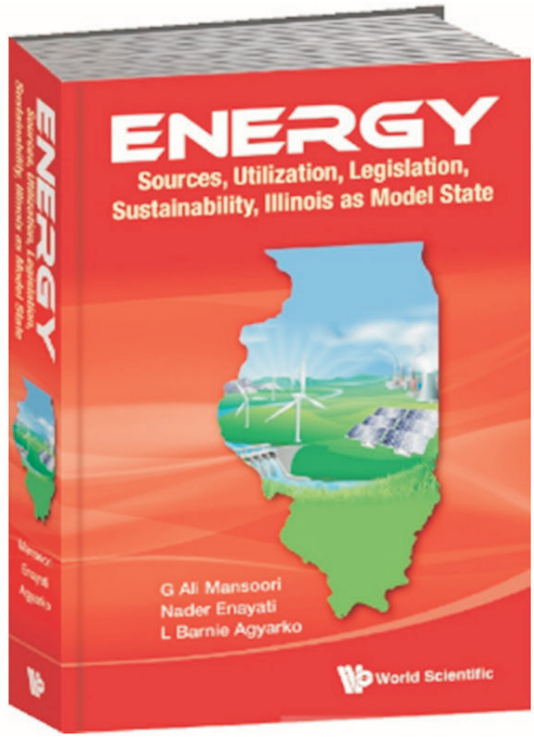
ENERGY: Sources, Utilization, Legislation, Sustainability, Illinois as Model State

Authored by G.A. Mansoori, N. Enayati and B. Agyarko; World Scientific Publishing Co., Hackensack, NJ, 810 pages (January 2016). (Hardcover: $(US) 214.00, ISBN 978-981-4704-00-7); (Ebook: $(US) 171.00. ISBN 978-981-4704-02-1)



This new book authored by Prof. GA Mansoori and his co-authors aims to ease understanding of the general public of energy storage and conversion methods, environmental concerns associated with energy conversion, economics and future trends of energy conversion technologies. The book is written in an excellent fashion, partly in technical language (reporting mathematical equations and chemical formulae, flow charts, graphs and tables) of interest to scientific and engineering community, and partly in non-technical language to make it easy to understand by non-technical communities. All the energy sources including fossil fuels, nuclear, wind, solar, geothermal and biomass along with energy storage methods are discussed and all the real examples of energy conversion and storage methods are illustrated. Examples of central governments, state and local governments and private businesses involvement in promoting energy efficiency and environmental protection are shown to illustrate effectiveness of collective action. Of course, considering that the State of Illinois, USA, has been a pioneer in all aspects of energy it is used as a model state in this book. State of Illinois is where the peaceful use of nuclear energy was first utilized to produce electricity, the state with biggest user of coal and pioneer in conversion of energy uses to renewable sources.

It is interesting and quite significant to note that this book is quite timely published, immediately after the UNFCCC COP 21, also known as the 2015 Paris Climate Conference, held last December in Paris, France. Nearly 200 countries, including Pakistan and USA signed the COP21 Climate Agreement on December 12, 2015. This historic agreement aimed to reduce, or even prevent, environmental effects of global warming by reducing release of greenhouse gases into the atmosphere. This marvelous book covers all the possible ways to achieve the aims of the COP21 Climate Agreement.

An introductory chapter which is open access by publisher for everyone to read and get familiar with the excellent contents of the book is available at: (*www.worldscientific.com/doi/suppl/10.1142/9699/suppl_file/9699_chap01.pdf*).

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