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Genetic Improvement of Tomato (*Solanum lycopersicum* L.) for Phytonutrient Content at AVRDC - The World Vegetable Center

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ABSTRACT

Tomato is a widely consumed global vegetable and a major source of the phytonutrients vitamin C, beta-carotene (provitamin A), lycopene, and flavonoids. Tomato cultivars with increased fruit phytonutrient density could help overcome micronutrient malnutrition and contribute to better human health. It is important for plant breeders to understand the genetic diversity and genetic control of targeted phytonutrients, the extent to which environmental factors such as temperature or light intensity affect phytonutrient content, and whether altered phytonutrient content significantly affects yield and horticultural or fruit quality traits. Tomato breeding at AVRDC - The World Vegetable Center has included phytonutrient objectives in its breeding programs and has developed tropically adapted, high yielding and multiple disease resistant lines with increased content of beta-carotene, lycopene, flavonoids, or anthocyanin in different fruit types. Increased content of some phytonutrients such as lycopene is associated with better fruit quality. On the other hand, high beta-carotene content results in orange-fleshed fruit, which is not readily accepted by many consumers. AVRDC seeks to popularize high phytonutrient tomato cultivars through linkages with organizations promoting nutrition and health.

Keywords: Nutrition, beta-carotene, lycopene, flavonoids.

Introduction

Micronutrients including vitamins and minerals are essential components of healthy diets and are required for proper physical and mental growth and immune system functions. However, it is estimated that more than two billion people worldwide are stunted, anemic, and or vitamin A deficient due to one more micronutrient deficiencies a condition also known as 'hidden hunger' (Muthayya *et al*., 2013). Vegetables are major dietary sources of micronutrients and provide antioxidant phytochemicals that act to prevent or neutralize free radical chain reactions, which may lead to development of cardiovascular diseases or cancers (Keatinge *et al*., 2013). AVRDC-The World Vegetable Center (AVRDC) is the leading international center for vegetable research and development with the mission of alleviating poverty and malnutrition in developing countries. Conventional plant breeding to increase density of phytonutrients (micronutrients and antioxidant phytochemicals) of selected vegetables crops is among AVRDC's multiple strategies to improve access of the poor to adequate supplies of safe and affordable vegetables (Yang *et al*., 2007).

Tomato is one of the most economically important, widely grown and consumed vegetables in the world (FAO, 2013). Tomato fruit contains significant amounts of beta-carotene (provitamin A carotenoid), lycopene, ascorbic acid, some phenolic acids and flavonoids, all of which can be phytonutrients. Although phytonutrient levels in tomato are small compared to some other vegetables (Keatinge *et al*., 2011), daily consumption of tomatoes is high in many countries and even modest increases in tomato phytonutrient content could contribute to better human health (Hanson *et al*., 2004).

Genetic variation for some phytonutrients exists in tomato. More than 20 genes have been described affecting the types and concentrations of carotenoids in tomato (Stommel, 2007). Some of these include the alleles *Beta* (B) and *crimson* (*og*^{c}) mapped to the Beta locus on chromosome 6 (Ronen *et al*., 2000); *high pigment-1* (*hp-1*) and *high pigment-2* (*hp-2*) mapped to chromosomes 2 and 1, respectively (Yen *et al*., 1997), as well as *hp-2*^{dg}, which is allelic to *hp-2* (Bino *et al*., 2005). Additional genes or quantitative trait loci (QTLs) have been identified that increase flavonoids such as the gene *Anthocyanin fruit* (*Aft*) mapped to chromosome 10, and a major QTL increasing rutin content mapped to chromosome 5 (Hanson *et al*., 2014). Tomato also produces phenolic acids including caffeic acid and chlorogenic acid that act as antioxidants (Bravo, 1998). Although there is potential to increase fruit nutrient content, tomato breeding programs have largely emphasized yield, disease resistance, and fruit quality objectives (Stommel, 2007). AVRDC tomato breeding assigns a high priority to increasing the content of some fruit phytonutrients, particularly carotenoids and flavonoids. Phytonutrients to include as breeding objectives are selected for their potential to make a significant improvement, the extent to which nutrients levels are affected by environmental factors, and prospects for acceptance by consumers and markets. This paper outlines some of the work on genetic improvement of tomato nutrition traits by AVRDC, the strategies used, and current progress.

Materials and Methods

Season-Year Effects on Phytonutrient Contents

Twelve tomato entries (Table 1) representing different fruit market types or carrying genes affecting fruit carotenoids, vitamin C, and anthocyanin content were grown at AVRDC Taiwan in two seasons for two years. Mean temperatures, rainfall, and solar intensities during trials are given in Table 2. Seasons 1 and 2 are early and late dry seasons, and conditions in season 1 usually favor tomato production compared to season 2. Plots included four or nine plants and entries were replicated three times and arranged in a randomized complete block design (RCBD). Plants were staked and pruned and grown using recommended management practices. Full red ripe fruit were harvested from the inner two or seven plants and provided to AVRDC Nutrition for analysis of phytonutrients except for vitamin C, which was not analyzed in year 1-season 1. Methods for phytonutrient analysis are given in Hanson *et al*., (2004). Data were subjected to analysis of variance for individual environments and over seasons and years using the general linear models (PROC GLM) procedure of SAS Online Version 9.4 software (SAS Institute, Inc., Cary, N.C.). For the combined analysis of variance, a mixed effects model was applied in which entry was considered a fixed effect and year, season, and replications were deemed random effects. The significance of mean squares was determined using appropriate error terms.

Dual-Purpose Tomato Trials

Two preliminary yield trials (PYT) were carried out at AVRDC-Taiwan in the first half of 2015. Both PYT included 8 test lines (coded CLN) and checks 'Tanya,' 'UC204A', and T5020. 'Tanya' and 'UC204A' are processing tomato cultivars and T5020 is a fresh market line homozygous for *hp-1* and *og*^{c}. PYT1 entries were sown and transplanted, respectively, on 2 and 30 December 2014. PYT2 entries were sown and transplanted, respectively, on 6 February and 10 March 2015. PYT1 plots consisted of two 1.5 m-wide beds with one 5.0-m-long row per bed (24 plants). PYT2 plots included 24 plants but consisted of one 1.5 m-wide bed with two 5.0-m-long rows per bed. Plants in PYT1 were not staked while PYT2 plants were staked and pruned. Entries were replicated twice and plots were arranged in a RCBD. PYT1 plots were harvested three times (9, 20, 30 April 2015). PYT2 plots were harvested on 20 May, 28 May, and 8 June. Fruit samples were taken to the AVRDC Nutrition lab for analysis of vitamin C, lycopene and beta-carotene. A separate and a combined analysis of variance were performed. Average daily maximum, minimum, and mean temperatures from transplanting to final harvest during PYT1 were 26.2 °C, 15.9 °C, and 20.2 °C respectively; total rainfall and average relative humidity during PYT1 were 109 mm and 68.1%, respectively, and average daily solar intensity was 5087 watt-hours per m^2. During PYT2, average daily maximum, minimum, and mean temperatures from transplanting to final harvest were 30.2 °C, 20.9 °C, and 24.8 °C respectively. Total rainfall and average relative humidity during the trial were 491 mm and 68.9%, respectively. Average daily solar intensity was 5798 watt-hours per m^2. Data were subjected to analysis of variance using SAS software.

Results

Season-Year Effects on Phytonutrient Content

The analyses of variance revealed highly significant entry means squares for all phytonutrients and quality traits except caffeic acid (Table 3). Season affected phytonutrient contents and are indicated by highly significant or significant season means squares

for all traits except beta-carotene. All entry-season interactions were nonsignificant for all traits except for beta-carotene; entry-year interactions were also nonsignificant except for lycopene and caffeic acid.

Carotenoids and Vitamin C

Mean lycopene content over entries was about 18% lower in season 2 compared to season 1 (Table 4a). Entries T5020 (*hp-1*+*og*^{c}) and ASVEG20 produced relatively high lycopene content in most trials. The three entries homozygous for *og*^{c} produced higher than average lycopene but were not outstanding compared to processing check 'UC204A.' Lycopene content of high anthocyanin line CLN3339FA was slightly lower but similar to other fresh market entries. Mean beta-carotene content was about 20% lower in season 1 versus season 2. As expected, the two entries homozygous for *Beta* developed about 5-8 times more beta-carotene content than the fresh market (Savior, CLN2498D) and processing ('UC204A') checks. T5020 and ASVEG20 developed about twice as much beta-carotene compared to the checks. Significant differences between entries were found for vitamin C content although only a two-fold range in entry means was found. ASVEG20 ranked as highest or second highest in vitamin C content in each trial.

Phenolics and Rutin (flavonoid)

Average caffeic acid content (Table 4b)varied greatly by both year and season; for example, mean caffeic acid content over entries was six times greater in year 1-season 1 compared to year 2-season 1. Similarly, entry means varied greatly between trials and were often inconsistent: CLN3339FA, for example, produced the highest caffeic content in two trials but levels were average or below-average in the other trials. Mean chlorogenic acid content was about 33% greater in season 2 versus season 1. Mean rutin content was highest in year 1-season 2 with relatively high mean temperatures and rainfall that were more stressful and likely induced higher flavonoids. Rutin content of entry BMZ51 varied among trials but was four or five times greater than other entries. CLN3339FA, CLN2366A, T5020 and ASVEG20 also developed relatively high rutin content.

Dual-Purpose Tomato Trial

Highly significant differences among entries were detected for all traits except lycopene content (Table 5). Optimal weather for tomato production and low TYLCD incidence likely accounted for the high mean marketable yield in PYT1 (95 t/ha). Mean yield in PYT2 (18 t/ha) was sharply lower because of strong tomato yellow leaf curl disease (TYLCD) pressure coupled with higher temperatures and rainfall. Even though there were no significant differences among entries in lycopene content in both trials, entries homozygous for the *og*^{c} allele usually produced high color values sometimes exceeding 2.0 (data not shown); this indicated presence of a deep red fruit color desirable for many consumers and by tomato processors. The three entries homozygous for *hp-1* showed relatively higher levels of beta-carotene and vitamin C.

Discussion

Consumption of vegetables and fruit provide essential micronutrients and phytochemicals that reduce risks of micronutrient deficiencies as well as chronic diseases such as atherosclerosis and cancers. Unfortunately, vegetable consumption in most of the tropics and subtropics falls far short of the minimum of 200 g per person per day (Keatinge *et al*., 2011). Action must be taken to increase vegetable supplies through better production and postharvest practices and to raise consumer demand for and consumption of vegetables. AVRDC-The World Vegetable Center has developed technologies to increase supplies of diverse and affordable vegetables, such as diversification of vegetable crops, especially indigenous vegetables; off-season vegetable production; intensive home gardening; improved postharvest practices; and optimized food preparation methods, all which ultimately contribute to better diets and human nutrition (Yang *et al*., 2007). Tropically adapted, high yielding vegetable cultivars coupled with sound management practices play an essential role in boosting vegetable supplies. Successful cultivars must meet the requirements of different value chain stakeholders and combine multiple traits, including high yield, multiple disease resistance, and long shelf-life, and be safe, nutritious and good tasting for consumers. Development of phytonutrient-dense cultivars is a worthy objective pursued by all AVRDC breeding programs but the breeding strategy must be designed and carried out with all end-users in mind.

Tomato is a globally popular vegetable consumed fresh, cooked, or in processed products. Consumption is high, and thus tomato fruit is an important source of carotenoids, vitamin C and flavonoids in diets. Manipulating carotenoid content in tomato is relatively straightforward because major genes affecting the types and levels of particular carotenoids are known and characterized (Ronen *et al*., 2000; Stommel, 2007). Vitamin A deficiency is a preventable cause of blindness in children and increases risks of some infectious diseases. In response, AVRDC initiated breeding to increase tomato beta-carotene

content in some tropical tomato lines using the *Beta* allele almost 20 years ago with funding from the Thrasher Foundation, USA. Presence of *Beta* boosted beta-carotene content by 3-9 times compared to normal red-fruited tomato (Yang *et al*., 2007). Beta-carotene contents of some early AVRDC *Beta* lines reached 9.0 mg/100 FW but such high levels resulted in a bitter aftertaste. Targets shifted to beta-carotene levels 3-5.0 mg/100g FW, which are still relatively high but do not noticeably affect taste. AVRDC *Beta* lines in fresh market and cherry tomato fruit types have been distributed internationally and have been officially released as cultivars in Mali, Taiwan, and Bangladesh (unpublished data). However, adoption so far has not been high because markets and consumers are unaware, unfamiliar, or reluctant to accept the orange fruit color. With low demand, financial incentives for seed companies or public organizations to produce of *Beta* tomato lines are weak. AVRDC *Beta* cherry tomato lines are tasty, productive, easy to grow, and perhaps best targeted for tropical home gardens. More partnerships with health and nutrition public organizations are needed to increase awareness and use of *Beta* tomato by gardeners.

Tomato cultivars originally developed for processing such as 'Roma VF', 'Rio Grande', 'UC82' and others are widely grown in the tropics for the fresh market and sometimes for processing (dual-purpose). Besides fruit firmness, dual-purpose tomato cultivars typically develop deep red internal color due to high lycopene content. The *hp-1* allele is interesting because it acts to elevate total carotenoid content without changing the proportions of lycopene and beta-carotene, and it also increases content of vitamin C and flavonoids; full-ripe fruit becomes red or sometimes red-orange. Gains in beta-carotene content with *hp-1* are not as great as *Beta,* but market and consumer acceptance would be far easier, and such lines could become popular if incorporated into firm fruit types and combined with TYLCD and other disease resistances with moderate to high levels of heat tolerance. High pigment lines CLN3669A (AVTO1418) and CLN3670B (AVTO1420) are TYLCD resistant and available through the AVRDC seed catalog (*http://avrdc.org/seed/improved-lines/*). AVRDC will continue to develop tomato lines with *hp-1* alone and in combination with og^c.

Evidence suggests that flavonoids are powerful antioxidants that can benefit human health (Ross and Kasum, 2002). Rutin is the major flavonoid in tomato and AVRDC identified a major QTL in a breeding line derived from an introgression line that increased rutin content by 4-5 times (Hanson *et al*., 2014). AVRDC is incorporating this QTL into elite tomato lines by marker-assisted selection and by developing near-isogenic lines (NIL) with or without the QTL to test whether high rutin content enhances disease resistance and/or abiotic stress tolerance. AVRDC trials indicated that levels of caffeic acid and chlorogenic acid were strongly affected by the environment and large entry rank changes were common; breeding to enhance these phenolics is not warranted. Vitamin C is important both as an essential nutrient and as an enhancer of iron bioavailability (Teucher *et al*., 2004). Presently, AVRDC characterizes its inbred lines for vitamin C content but has not actively bred to improve this trait, mainly because analysis is time-consuming and AVRDC has not yet methodically screened its genebank accessions for sources of high vitamin C. Genetic variation for vitamin C content was found in cultivated tomato, but environmental effects were also large (Leiva-Brondo *et al*., 2012). Availability of cheap, accurate, and fast vitamin C screening methods would facilitate breeding.

There is significant scope to genetically improve tomato through conventional breeding for a number of important phytonutrients, including carotenoids, vitamins, and flavonoids. Many major genes affecting nutrient content in tomato are well-known, but often not used in breeding. Breeding to increase phytonutrient content is most active when phytonutrient and fruit quality objectives are in alignment: tomato breeders have selected for increased lycopene content, which deepens internal red fruit color, a trait appreciated by processors and many consumers; lycopene was later determined to be a powerful antioxidant (Stommel, 2007). Few tomato breeding programs overtly include nutrition objectives. AVRDC breeding programs pursue nutrition objectives to support the Center's mandate to alleviate poverty and malnutrition. Mounting consumer demand for nutritious and safe vegetables may create market incentives to drive more tomato breeding programs to take up nutrition.

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Table 1. Entry names and genes affecting fruit phytonutrient contents evaluated at AVRDC Taiwan.

Entry name	Origin	Allele (symbol) or comment	Effect of fruit phytonutrient gene	Reference
CLN3339FA	AVRDC	Anthocyanin fruit (*Aft*)	Increased anthocyanin in fruit skin is expressed if fruit not shaded	Jones *et al*., 2003 Sapir *et al*., 2008
CLN2366A	AVRDC	Beta (B)	Elevated fruit beta-carotene content at expense of lycopene	Tomes *et al*., 1956
CLN2070A	AVRDC	Beta (B)	Elevated fruit beta-carotene content at expense of lycopene	Tomes *et al*., 1956
BMZ51F2-67-1-30 (BMZ51)	AVRDC	High flavonoid QTL	Elevated rutin in fruit pericarp	Hanson *et al*., 2014
CLN3070J	AVRDC	*crimson* (*og^c*)	Elevated fruit lycopene content	Ronen *et al*., 2000
CLN3125P	AVRDC	*crimson* (*og^c*)	Elevated fruit lycopene content	Ronen *et al*., 2000
NCEBR-6	USA	*crimson* (*og^c*)	Elevated fruit lycopene content	Ronen *et al*., 2000
T5020	USA	*crimson* (*og^c*) and high pigment (*hp-1*)	Elevated carotenoids, vitamin C, and flavonoids	Wann, 1997
ASVEG20	AVRDC	Dark green fruit pericarp, presumed to be due to action of *dark-green* (*dg*)	Elevated carotenoids, vitamin C	Levin *et al*., 2003 Konsler, 1973 Bino *et al*., 2005
Savior	Syngenta	Fresh market hybrid (check)		
CLN2498D	AVRDC	Fresh market line (check)		
UC204A	USA	Processing cultivar (check)		

Table 2. Weather data summary of tomato trials conducted to assess phytonutrient content and other fruit traits of selected tomato lines over two seasons and two years, AVRDC Taiwan, 2011-2013.

Trial	Date			Mean daily air temperature (°C)		Total precipitation	Mean daily solar intensity
	Sowing	Transplant	Harvest	Max	Min	mm	(w-h W ·m^2)
Year 1 - Season 1	26 Oct 2011	26 Nov	3 Apr	24.2	15.4	59	3633
Year 1 - Season 2	9 Feb 2012	20 March	8 June	32.2	21.7	431	5572
Year 2 - Season 1	10 Sept 2012	9 Oct	26 Dec	28.9	17.7	103	4172
Year 2 - Season 2	24 Jan 2012	27 Feb	9 May	29.5	18.4	136	4838

Table 3. Significance of means squares from the combined analysis of variance of phytonutrients over two years and two seasons, AVRDC Taiwan.

Source of Variation	Degrees of Freedom	Lycopene	Beta-carotene	Caffeic acid	Chlorogenic acid	Rutin
Year (Yr)	1	–	–	–	–	–
Season (Yr)	2	**	ns	**	**	**
Rep (Yr*Seas)	8	–	–	–	–	–
Entry	11	**	**	ns	**	**
Entry*Yr	11	**	ns	**	ns	ns
Entry*Seas	11	ns	**	ns	ns	ns
Entry*Yr*Seas	11	ns	ns	**	**	**
Pooled	88	–	–	–	–	–

Source of Variation	Degrees of Freedom	Vitamin C
Environments (Env)	2	**
Reps (Env)	6	-
Entry	11	**
Entry*Env	22	**
Pooled	66	–

**=significant at P<0.01; ns=not significant

Table 4a. Entry means for carotenoids and vitamin C by season and year, AVRDC Taiwan.

Trial	Lycopene				Beta-carotene				Vitamin C			
	Season 1		Season 2		Season 1		Season 2		Season 1		Season 2	
	------mg/100 g fresh weight-----				------mg/100 g fresh weight-----				------mg/100 g fresh weight-----			
	Year 1	Year 2	Year 1	Year 2	Year 1	Year 2	Year 1	Year 2	Year 1	Year 2	Year 1	Year 2
CLN3339FA	4.86 e	5.87 a	4.95 cd	3.34 bc	0.22 e	0.39 cd	0.33 f-h	0.26 ef	-	24.9 b-d	19.0 c-e	17.8 de
CLN2366A	1.14 f	1.49 b	0.88 e	0.83 de	2.48 b	2.27 a	2.21 b	2.07 a	-	23.9 de	15.7 fg	18.5 c-e
CLN2070A	1.03 f	1.15 b	0.26 e	0.25 e	3.16 a	2.12 a	3.62 a	2.00 a	-	28.0 b	24.9 a	20.8 bc
BMZ51	6.09 c-e	6.82 a	6.51 bc	4.44 ab	0.49 de	0.40 cd	0.49 de	0.46 c	-	27.7 bc	20.8 bc	19.8 cd
CLN3070J	7.76 b-d	6.22 a	8.48 ab	5.21 ab	0.30 de	0.19 d	0.21 gh	0.15 g	-	24.3 cd	14.4 g	14.7 fg
CLN3125P	6.87 b-e	7.03 a	8.13 ab	4.24 ab	0.39 de	0.31 d	0.41 d-f	0.23 e-g	-	20.6 ef	18.9 c-f	17.5 de
NCEBR-6	6.15 c-e	5.71 a	5.02 cd	4.81 ab	0.32 de	0.22 d	0.16 h	0.18 fg	-	18.6 f	16.5 e-g	13.6 g
T5020	11.15 a	6.46 a	8.98 a	5.53 a	0.59 d	0.71 bc	0.55 d	0.41 c	-	32.0 a	20.2 cd	22.8 ab
ASVEG20	9.42 ab	5.88 a	9.00 a	4.21 ab	1.09 c	1.05 b	0.73 c	0.85 b	-	33.4 a	23.7 ab	23.7 a
Savior	7.39 b-e	5.96 a	7.70 ab	4.78 ab	0.36 de	0.25 d	0.32 f-h	0.30 de	-	27.7 bc	21.5 bc	18.8 c-e
CLN2498D	5.02 de	7.01 a	4.00 d	2.20 cd	0.62 d	0.42 cd	0.47 d-f	0.43 c	-	22.8 de	19.4 c-d	16.2 ef
UC204A	8.09 bc	6.52 a	7.26 ab	5.44 a	0.44 de	0.36 cd	0.33 e-g	0.39 cd	-	20.5 ef	17.2 d-g	19.1 cd
LSD (P=0.05)	2.75	2.12	2.11	1.90	0.27	0.37	0.17	0.09	-	3.5	3.2	2.6
Mean	6.24	5.51	5.92	3.77	0.87	0.82	0.72	0.64		25.4	19.4	18.6
Entry mean square significance	**	**	**	**	**	**	**	**		**	**	**

**=significant at P<0.01

Table 4b. Entry means for phenolic acids and rutin by season and year, AVRDC Taiwan.

Trial	Caffeic Acid				Chlorogenic acid				Rutin			
	Season 1		Season 2		Season 1		Season 2		Season 1		Season 2	
	------mg/100 g fresh weight-----				------mg/100 g fresh weight-----				------mg/100 g fresh weight-----			
	Year 1	Year 2	Year 1	Year 2	Year 1	Year 2	Year 1	Year 2	Year 1	Year 2	Year 1	Year 2
CLN3339FA	2.48 a	0.08 f	2.46 a	0.33 de	1.74 a-c	4.88 a	7.10 a	6.80 a	1.79 b-d	6.72 b	7.52 bc	5.91 b
CLN2366A	1.51 b	0.23 b	0.87 b-d	0.47 b	2.78 a	5.64 a	6.66 a	1.72 c-e	2.03 bc	4.74 c	9.00 b	4.14 bc
CLN2070A	2.29 a	0.37 a	0.79 b-e	0.44 bc	2.30 ab	4.44 a	4.07 b	0.85 e	2.06 bc	2.36 de	4.56 c-e	2.40 c-e
BMZ51	1.19 bc	0.18 cd	0.89 b-d	0.32 de	1.87 a-c	2.84 b	3.23 bc	2.42 c	9.05 a	16.3 a	37.03 a	15.68 a
CLN3070J	1.02 bc	0.14 de	0.48 d-f	0.12 g	1.25 b-d	1.95 bc	1.98 cd	2.35 c	0.69 d	0.90 e	1.61 e	0.86 e
CLN3125P	0.55 c	0.24 b	0.52 c-f	0.19 fg	1.10 b-d	1.20 c	1.87 cd	2.11 cd	1.06 cd	1.11 e	2.29 de	1.61 de
NCEBR6	0.88 bc	0.15 de	0.31 f	0.18 fg	0.99 cd	1.47 c	1.73 d	2.48 c	1.24 cd	1.78 de	2.97 de	1.37 de
T5020	0.96 bc	0.10 ef	0.93 bc	0.23 ef	0.24 d	2.19 bc	2.05 cd	1.27 de	1.87 b-d	2.51 de	6.40 bc	2.02 de
ASVEG20	1.32 b	0.39 a	1.01 b	0.35 cd	2.89 a	2.32 bc	4.35 b	3.79 b	2.65 b	2.19 de	5.01 cd	2.97 cd
Savior	0.59 c	0.26 b	0.43 ef	0.16 fg	1.20 b-d	1.63 bc	2.99 b-d	2.00 cd	1.09 cd	1.11 e	2.75 de	1.43 de
CLN2498D	1.07 bc	0.17 d	0.85 b-e	0.63 a	2.21 a-c	1.76 bc	3.00 b-d	1.22 de	2.17 bc	2.85 d	5.31 cd	2.28 de
UC204A	1.08 bc	0.10 ef	0.47 d-f	0.16 fg	2.70 a	1.67 bc	2.40 cd	2.43 c	1.22 cd	1.28 de	2.63 de	1.94 de
LSD (P=0.05)	0.65	0.06	0.43	0.10	1.22	1.31	1.42	1.06	1.29	1.68	3.25	1.79
Mean	1.24	0.20	0.83	0.30	1.77	2.67	3.45	2.45	2.24	3.65	7.26	3.55
Entry mean square	**	**	**	**	**	**	**	**	**	**	**	**

**=significant at P<0.01; ns=not significant

Table 5. AVRDC dual-purpose tomato lines evaluated for yield and fruit traits in PYT1 (December-April 2015) and/or PYT2 (March-June 2015) in Taiwan.

Entry	Genes affecting carotenoids		Marketable yield (t/ha)		Color (a/b)[1]		Vitamin C (mg/100 g FW)		Beta-carotene (mg/100 g FW)		Lycopene (mg/100 g FW)	
	high pigment (hp-1)	*crimson (og^c)*	PYT1	PYT2	PYT1	PYT2	PYT1	PYT2	PYT1	PYT2	PYT1	PYT2
Tanya	–	–	73	4	1.97	1.80	14.3	19.2	0.37	0.28	8.03	8.00
UC204A	–	–	87	10	1.81	1.63	18.2	23.1	0.36	0.40	7.49	7.28
T5020	+	+	55	4	2.14	1.65	20.6	27.6	0.56	0.53	8.67	8.37
CLN3682C	–	+	104	26	2.23	2.07	15.4	18.7	0.15	0.05	7.69	8.13
CLN3682A	–	+	89	22	2.07	1.89	19.6	20.2	0.23	0.19	7.89	7.30
CLN3682D	–	+	102	30	2.09	2.09	17.0	19.5	0.16	0.16	6.92	7.51
CLN3552B	–	+	133	21	2.18	2.01	19.4	24.0	0.59	0.34	7.18	9.85
CLN3552F	–	+	41	19	2.50	2.23	18.9	17.8	0.14	0.10	7.43	6.57
CLN3669A	+	–	130	24	1.84	1.86	33.4	42.7	0.86	0.62	7.99	9.04
CLN3670B	+	–	117	22	1.88	1.63	23.1	27.4	0.62	0.52	7.80	7.97
CLN3670F	+	–	127	19	1.72	1.77	23.3	25.9	0.77	0.45	7.83	8.27
Mean			95	18	2.02	1.87	20.1	24.2	0.43	0.33	7.55	8.02
Entry MS			**	**	**	**	**	**	**	**	ns	ns
LSD (0.05)			27	6	0.19	0.15	2.2	3.9	0.21	0.14	-	-
CV			13.0	15.6	4.28	3.70	12.1	7.3	22.6	19.05	18.3	24.45

**=significant at P<0.01; ns=not significant

[1]Values for a and b were measured with a chromometer using a red standard surface. Immature green tomatoes have a/b ratio less than 0. The a/b ratio increases to zero and above as the fruits ripen toward a dark red. Values > 2.0 have superior color.

+, – indicates presence or absence, respectively, of the allele

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Effects of Salt Stress on Vegetative Growth Parameters and Ion Accumulations in Cucurbit Rootstock Genotypes

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ABSTRACT

Salt stress leads to decreases in plant growth, development, yield and quality changes of many plant species. Winter squash and pumpkins were recommended for use of rootstocks for the grafted watermelon, melon and cucumber growing in the saline soils. Grafted seedlings recently are being used widely for vegetable crops grown in many countries of the world. In this study, it was aimed to identify differences in salt tolerance of local winter squash, bottle gourd, pumpkin genotypes, and their interspecific rootstock hybrids (*Cucurbita maxima x Cucurbita moschata*) by using some vegetative growth parameters and ion accumulations. Salt was applied at 4,8,12, and 16 dS m^{-1}NaCI salinity levels for each genotypes. Non-salt-treated plants were kept as controls. Plant vegetative growth parameters such as plant height, stem diameter, leaf number and leaf area were negatively affected by salt stress. The results showed that NaCI treatment caused an increase in Na+ ion concentration and decreased in K^+, and Ca^{++} ion concentrations. In conclusion, Cucurbit rootstock genotypes showed large variation in their response to salt tolerance. Seven pumpkin inbred lines (G2, G3, G4, G7, G29, G30, and G31), three winter squash inbred lines (G9, G12, and G13), three interspecific hybrids of *C. maxima* x *C. moschata* (G14, G15, and G40) were found as salt tolerant. We would highly recommend use of promising salt tolerant rootstock genotypes for grafted watermelon, melon and cucumber seedling production.

Keywords: winter squash, pumpkin, hybrid, rootstock, growth, resistance, salinity.

Introduction

Salinity is one of the most important abiotic stress factors that cause reduction in plant growth, development and yield values. Plant species can differ markedly in their responses to salt tolerance (Dasgan and Koc 2009; Kusvuran *et al*., 2011). In terms of salt resistance; there are differences between family, genera, species and significant differences between genotypes (Belkhodja *et al*., 1994). Most of vegetable crops are sensitive to salt stress and these can't survive under saline conditions. Salt stress changes the plant's morphological and physiological traits and biochemical responses (Sevengor *et al*., 2011; Kusvuran *et al*., 2013). The plants have lower growth rates and their leaves are mostly small, with a dark green color in salt stress (Greenway and Munns 1980). In the presence of excess salt during plant growth Na^+ and Cl^- are accumulated in different plant organs (Levitt 1980; Kurtar *et al*., 2016). Many researchers have reported that long term salinity stress causes ion toxicity, water deficiency in older leaves and occurrence of the carbohydrate deficiency in young leaves (Greenway and Munns 1980; Franco *et al*., 1993; Tipirdamaz and Ellialtioglu 1997; Demir 2009; Kuşvuran 2010; Kurtar *et al*., 2016). Therefore, salt resistance often depends on the ability of the plant to develop adaptive strategies under stress conditions (Kachout *et al*., 2012; Ors and Suarez 2016).

Winter squash and pumpkin species are members of the genus Cucurbita within the economically important *Cucurbitaceae* family. There are three economically important Cucurbita species, namely *Cucurbita pepo*, *Cucurbita maxima* and *Cucurbita moschata*, which have different climatic adaptations, and are widely distributed, in agricultural regions worldwide (Robinson and Decker-Walters 1997; Paris and Brown 2005; Wu *et al*., 2007; Balkaya *et al*., 2009; Balkaya *et al*., 2010). Winter squash and pumpkin are usually grown for their fruits i.e. immature for summer squash, and mature for the winter squash and pumpkin.

Cucurbit plants are grafted onto various rootstock species and varieties using a range of grafting methods. Cucurbit crops that are commonly grafted include watermelon, melon and cucumber. The most common rootstocks for watermelon are bottle gourd, interspecific hybrids between *C. maxima* and *C. moschata* and wild watermelon (*C. lanatus* var. *citroides*) (Davis *et al*., 2008; Karaagac and Balkaya 2013). The compatibility of watermelon with any of these rootstocks is generally high, although there is variability within the species (Yamamuro and Marukawa 1974; Karaagac 2013; Gungor and Balkaya 2016). The most commonly used *Cucurbita* spp. rootstock is interspecific *C. maxima* × *C. moschata* hybrid (Colla *et al*., 2010). The use of rootstocks has been shown to enhance the vigor of the scion through the resistance to soil pathogens and tolerance to low soil temperatures and/or salinity (Ruiz *et al*., 1997). The use of rootstock is a valid strategy in increasing salt tolerance by reducing sodium toxicity.In a research, interspecific *C. maxima* × *C. moschata* hybrids were found as resistant to salt stress. *C. moschata* and *Lagenaria siceraria* genotypes showed tolerant level resistance against to salt stress (El-Shraiy *et al*., 2011).

In terms of salt tolerance, genotypic variations were found by Sevengor (2010) between local squash and pumpkin cultivars in Turkey (Balkaya and Kandemir 2015). Winter squash and pumpkin can be grown on unproductive land without irrigation in many regions of Turkey. Therefore, winter squash and pumpkin growing can be considered as a suitable alternative for the problem of salinity or drought in areas (Sevengor *et al*., 2011; Kurtar *et al*., 2016).

Grafting onto salt-tolerant rootstock is an effective method for increasing the salt tolerance of plants. Grafting has been found to improve the salt tolerance of tomato (Estan *et al*., 2005; Santa-Cruz *et al*., 2002), eggplant (Wei *et al*., 2007; Curuk *et al*., 2009), watermelon (Yetisir and Uygur 2010; Gungor and Balkaya 2016), melon (Edelstein *et al*., 2005; Dasgan *et al*., 2015), and cucumber (Zhu *et al*., 2008). Grafting can raise the salt tolerance of watermelon and melon (Yetisir and Uygur 2010; Dasgan *et al*., 2015). The aim of this study was to identify differences in salt tolerance of local winter squash, bottle gourd, pumpkin genotypes and their interspecific *C. maxima* x *C. moschata* rootstock hybrids by using some vegetative growth parameters and ion accumulations.

Materials and Methods

Materials: In this study, 17 inbred winter squash lines, 20 inbred pumpkin lines, 7 interspesific rootstock hybrids (*C. maxima* x *C. moschata*) and Shintoza F_1, Obez F_1 rootstock cultivars, 1 bottle gourd genotype (*Lageneria siceraria*), and one pumpkin cultivar (cv. Titan) were used (Table 1). These genetic materials, consisting of winter squash and pumpkin lines were developed at the S5-S6 generation, and interspecific rootstock hybrids between *C. maxima* and *C. moschata* rootstock were also obtained from breeding program for grafted watermelon by Balkaya *et al*., (2011) and Karaagac (2013).

Growth condition: This study was carried out in the controlled plant growth cabin of the Department of Horticulture, during 2013-2014. Seeds were germinated in a mixture of peat: perlite of 2:1 ratio. After 14 days of sowing, seedlings were transferred to plastic pots (7 l volumes) containing perlite. The nutrient solution utilized a modified Hoagland's solution (9 g/l$Ca(NO_3)_2$; 2.5 g/lK_2SO_4; 4.5 g/l $MgSO_4$; 2 g/l KH_2PO_4; 0.035 g/l H_3BO_3; 0.015 g/l $MnSO_4$; 0.01 g/l $CuSO_4$; 0.012 g/l $(NH_4)_6Mo_7O_{24}$; 0.02 g/l $ZnSO_4$, 0.3 g/l Fe EDTA), and it was renewed every 3 days. The base nutrient solution without added salts served as control in this study.

Salt treatment: Salt solution treatment application was started when the seedlings have reached at 4-5 true leaf stage. Sodium chloride was used as salt resource. Salt treatments were applied 4 different EC values (4, 8, 12, 16 dS m^{-1}). Non-salt-treated plants were kept as controls. After the salt treatment, all pots were covered with aluminum foil to prevent loss of salt by evaporation.

Plant vegetative growth parameters: At the end of 30 days after salt treatment, stress responses of experiment genetic materials were evaluated using by some plant vegetative growth parameters such as plant height, stem diameter, leaf number, leaf area,

shoot dry weight, root dry weight (Kusvuran 2010). All genetic materials were also classified for their salt tolerance according to leaf damage symptoms by using 0-5 scale symptom scores (Yildiz 2014). Control plants and undamaged plants were defined as "0" value.

Determination of ion contents: Ion contents (Na^+, K^+ and Ca^{++}) were determined according to Kacar (1984). For the ion determination, the plants were separated into shoot and leaves. These plant sections were dried at 70°C for 48 h. Na^+, K^+ and Ca^{++} concentrations were measured by using FLAME spectrometer.

Statistical analysis: The experimental design was randomized plot. Each treatment was replicated three times with ten plants. The results were analyzed using JUMP 5.0.1, and the mean values were compared using the least significant difference test ($P<0.05$).

Results and Discussion

In this study, salt stress treatments have caused various effects in all Cucurbit rootstock genotypes. The first characteristic response of plants under salt toxicity has shown a significant decrease in vegetative growth. According to the results, the plant height was significantly decreased compared to control plants with the increasing salt concentration doses in all Cucurbit rootstock genotypes (Table 2). The highest decrease was recorded in G40 interspecific hybrid as 96.3% (Table 2). The least decrease among these genotypes were determined as G9 (30.3%), G8 (41.4%) and G15 (43.7%), respectively. This decrease values was changed from 83.0 to 89.2% ratios between Shintoza F_1 (G32) and Obez F_1 (G33) rootstock cultivars. At the end of this study, plant height values were found at lower levels in all genotypes under salt stress compared to control treatment (Table 2).

After the salt treatment, terminal and edges of older leaves turn yellow along with the slowdown in plant growth. After that, this situation continues in the form of leaf chlorosis by moving towards the main xylem vessels and at later stage chlorosis is transformed into necrosis. Necrosis causes drying in leaf (Bergmann 1992; Ertekin 2010). In this study, the number of leaves in all genotypes was decreased under salt stress treatments. These values were found between 0- 80.3% amongst Cucurbit rootstock genotypes (Table 2). The highest reducing ratios were found in G17 (80.3%), G16 (77.0%) genotypes and Shintoza F_1 cultivar rootstock (74.5%) for the leaf number trait. Under salt stress condition, the least affected genotypes were determined as G12 (0%), G3 (9.5%) and G2 (12.2%) lines, respectively.

The leaf area values of all Cucurbit genotypes were decreased with salt treatments. The highest decrease was observed in G16 (92.9%), G20 (92.3%), and G43 (91.4%), respectively (Table 3). The leaf area decreasing ratio of Shintoza F_1 and Obez F_1 rootstock cultivars were changed from 73.0% to 81.1%. In this study, the effect of salinity in Cucurbit rootstock genotypes were generally apparent as all reduced vegetative growth parameters. These plants had possessed smaller leaves and sometimes fewer leaves.

At the end of salt stress (16 dS m^{-1}), Na^+ ions values were increased in all Cucurbit rootstock genotypes (Figure 1). In this study, the least increasing value of Na% were observed respectively in G9 (420.0%), G15 (433.3%), G5 (520.0%) and G3 (525.0%) genotypes compared to the control treatment. These genotypes have been found more selective in terms of salt ion content. In contrast, genotypes G31, G28, G19 and G29 accumulated a relatively larger quantity of Na^+ ions in to their texture. Munns (2002) reported that salt resistance plants have received Na and Cl ions to their texture at lower rates according to sensitive plants. Yetisir and Uygur (2009) also mentioned which Cucurbita and Lageneria rootstocks developed some mechanism to avoid physiological damage caused by excessive accumulation of Na^+ ion in leaves and shower higher performance than watermelon under salinity stress.

K^+ ion contents of Cucurbit rootstock genotypes showed large variation (Figure 2). Genotypes G5 (9.7%), G14 (12.2%), G3 (18.0%) and G30 (20.8%) had the maximum protecting ability of their K^+ ion content compared to control. In some genotypes, significant decrease in K ion content was found under salt stress conditions. The highest decrease in value of K ion content was determined in G41 genotype (81.6%). Shintoza F_1 and Obez F_1 rootstock cultivars also showed similar results.

Plants provide their balance with the help of inorganic ions under salt stress. The osmotic potential in the cell increases and more water can enter to the cell by taken K^+ with active absorption and accumulation in plants (Koc 2005; Kusvuran 2010). Therefore, K^+ content in the cell is important for maintenance of osmotic equilibrium. Romero *et al*., (1997) reported that increasing Na^+ concentration in leaves causes K^+ deficiency due to antagonist effect of Na^+ and K^+ ions.

Calcium is an important element to maintain cell membrane integrity and provide selectivity of ion

intake and transportation. Higher salt concentrations caused to reduce intake of Ca^{+} ion and ion imbalance in plant. (Cramer *et al*., 1986; Huang and Redman 1995). Calcium ion contents under salt stress are given in Figure 3. Calcium ions of all Cucurbit genotypes decreased under salt stress. The highest reductions were determined in G15 (13.7%), G28 (14.1%), G9 (18.4%) and G7 (23.0%) genotypes, respectively. These values were found 67.9% for Obez $F_1$and 30.4% Shintoza F_1.

K^{+}/Na^{+} and Ca^{++}/Na^{+} ratio were calculated due to Na^{+}, K^{+} and Ca^{++} ion contents. These results are shown in Figure 4 and Figure 5. K/Na and Ca/Na ratios decreased with the increasing salt concentration in all Cucurbit rootstocks genotypes. Kusvuran(2010) mentioned that decrease in Ca/Na ratios effected plant growth negatively. Similar results were found in this study.

According to the combined results of vegetative growth parameters and ion analysis; seven pumpkin inbred lines (G2, G3, G4, G7, G29, G30, and G31), three winter squash inbred lines (G9, G12, and G13), three interspecific hybrids of *C. maxima* x *C. moschata* (G14, G15, and G40) were selected as salt resistant genotypes for rootstock breeding program.

Conclusion

Salinity is a major abiotic stress factors limiting crop production. Winter squash and pumpkins that can be grown without irrigation are a good alternative for the soils with salinity problems in arid and semi-arid ecology. The use of rootstocks has been shown to enhance tolerance to salinity. Grafting onto salt-tolerant rootstock is an effective method for increasing the salt tolerance of plants. In this study, winter squash, pumpkin, their interspecific hybrids, and bottle gourd genotype were exposed to salt stress at increasing EC levels (4, 8, 12, and 16 dS m^{-1}. According to obtained results, vegetative growth parameters such as plant height, stem diameter, the number of leaves and leaf area were significantly decreased, but the number of dry leaves were increased under salt stress. Na^{+} accumulation has played an important role against salt resistance. Na^{+} increased in all Cucurbit genotypes depending on the salt treatments. It was found that sensitive genotypes had higher amount of toxic ion accumulation under salt stress. These results also showed that salt tolerant Cucurbit rootstock genotypes had taken More K^{+} and Ca^{++} ions selectively than the remaining other genotypes. At the end of this study, seven pumpkin inbred lines (G2, G3, G4, G7, G29, G30, and G31), three winter squash inbred lines (G9, G12, and G13), three interspecific hybrids of *C. maxima* x *C. moschata* (G14, G15, and G40) were found as salt tolerant. These findings suggest that selected promising salt tolerant rootstock genotypes will be used for grafted watermelon, melon and cucumber seedling production in near future.

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Table 1. Accession number and work codes of the used genetic materials in the experiment.

Species/Cultivar	Work Code	Accession Number
Pumpkin	G1	B15
	G2	BE11
	G3	BA10
	G4	BY13
	G5	B4
	G6	BM15
	G7	B1
	G16	MOE 1
	G17	MOE 2
	G18	MOE 3
	G22	14 BO 01
	G27	14 BO 03
	G28	05-19
	G29	05-14
	G30	Sarı-01
	G31	Pembe-05
	G44	05 ME 11
	G45	19 İS 06
	G46	05 AM 02
	G51	55NE01
Winter squash	G8	BLHO
	G9	K4
	G10	K25
	G11	Gode
	G12	K6
	G13	K13
	G19	MAE 2
	G20	MAE 3
	G21	MAE 4
	G23	55 ÇA 06
	G24	55 ÇA 15
	G25	55 BA 03
	G26	57 Sİ 21
	G47	05 AM 08
	G48	05 AM 02
	G49	57 AY 01
	G50	57 Sİ 03
Bottle gourd	G36	55BA01
Interspecific hybrids (*C. maxima* x *C. moschata*)	G14	9X14 I
	G15	3X14
	G39	M12XEXC
	G40	07XSE
	G41	NSUX09
	G42	BOX02
	G43	UNRX04
Rootstock cultivars	G32	Shintoza F_1
	G33	Obez F_1
Pumpkin cultivar	G38	Titan

Table 2. Changes in plant height, leaf number and stem diameter of Cucurbit genotypes under salt stress.

Genotype	Plant height (cm)			Leaf number (unit)			Stem diameter (mm)		
	0 dS m^{-1}	16 dS m^{-1}	Diff. (%)	0 dS m^{-1}	16 dS m^{-1}	Diff. (%)	0 dS m^{-1}	16 dS m^{-1}	Diff.(%)
G1	68.33 g-k[a]	18.67 f-j	-72,7	12.33 g-o	7.33 b-g	-40,6	6.36 klm	5.18 fgh	-18,6
G2	120.67 bc	40.67 abc	-66,3	13.67 d-l	12.00 a	-12,2	6.09 l-o	5.84 efg	-4,1
G3	98.00 b-g	31.00 b-f	-68,4	14.00 d-k	12.67 a	-9,5	6.55 j-m	5.82 efg	-11,2
G4	90.00 c-h	28.00 c-f	-68,9	13.00 f-n	8.67 bc	-33,3	10.43 b-e	6.25 def	-40,1
G5	97.67 b-g	45.00 a	-53,9	13.67 d-l	8.67 bc	-36,6	6.89 i-m	5.84 efg	-15,2
G6	99.00 b-g	41.33 ab	-58,3	17.33 b-e	13.66 a	-21,2	6.24 lmn	5.86 efg	-6,1
G7	57.33 i-n	30.00 b-f	-47,7	10.33 k-q	9.00 b	-12,9	8.89 d-g	6.62 c-f	-25,5
G8	34.67 l-q	20.33 e-i	-41,4	11.66 i-o	7.33 b-g	-37,1	10.45 b-e	9.32 a	-10,8
G9	66.00 h-l	46.00 a	-30,3	9.66 l-q	8.00 b-e	-17,2	9.95 c-f	8.13 abc	-18,3
G10	55.00 i-o	27.33 def	-50,3	11.33 j-p	6.67 c-i	-41,1	7.50 g-l	6.32 def	-15,7
G11	53.00 j-o	25.67 d-h	-51,6	14.00 d-k	9.00 b	-35,7	9.04 d-g	5.86 efg	-35,2
G12	77.67 e-j	29.67 b-f	-61,8	8.33 opq	8.33 bcd	0,0	9.74 c-f	7.15 b-e	-26,6
G13	85.67 d-i	34.67 a-d	-59,5	9.00 n-q	7.33 b-g	-18,6	8.85 e-h	7.68 a-d	-13,2
G14	79.67 e-j	33.00 a-e	-58,6	9.33 m-q	6.00 e-k	-35,7	10.66 b-e	7.16 b-e	-32,8
G15	74.67 f-j	42.07 ab	-43,7	10.00 k-q	7.11 b-h	-28,9	12.53 a	8.45 ab	-32,6
G16	76.67 f-j	3.67 k	-95,2	16.00 c-h	3.67 lm	-77,1	7.06 h-m	3.67 h-k	-48,0
G17	101.00 b-f	4.67 k	-95,4	27.00 a	5.33 g-l	-80,3	8.34 f-j	2.92 i-n	-65,0
G18	27.00 n-q	3.33 k	-87,7	13.33 e-m	4.00 klm	-70,0	6.66 i-m	2.46 k-o	-63,1
G19	113.33 bcd	6.67 jk	-94,1	16.33 c-g	7.63 b-f	-53,3	7.54 g-l	2.03 k-p	-73,1
G20	78.67 e-j	4.33 k	-94,5	11.33 j-p	4.00 klm	-64,7	9.50 c-f	2.57 j-o	-73,0
G21	118.33 bc	7.50 ijk	-93,7	12.00 h-o	4.33 j-m	-63,9	9.50 c-f	2.63 j-o	-72,3
G22	9.33 q	3.97 k	-57,5	12.00 h-o	5.67 f-l	-52,8	6.17 lmn	1.64 m-p	-73,4
G23	91.00 c-h	5.67 jk	-93,8	11.33 j-p	4.67 i-m	-58,8	9.25 c-g	3.24 i-m	-65,0
G24	101.00 b-f	7.00 jk	-93,1	11.67 i-o	4.00 klm	-65,7	10.71 a-d	2.14 k-p	-80,0
G25	99.33 b-g	7.17 jk	-92,8	13.00 f-n	5.33 g-l	-59,0	10.41 b-e	1.47 nop	-85,9

Continuing table 2

Genotype	Plant height (cm)			Leaf number (unit)			Stem diameter (mm)		
	0 dS m^{-1}	16 dS m^{-1}	Diff. (%)	0 dS m^{-1}	16 dS m^{-1}	Diff. (%)	0 dS m^{-1}	16 dS m^{-1}	Diff.(%)
G26	103.67 b-f	8.00 ijk	-92,3	14.00 d-k	5.00 h-m	-64,3	11.81 ab	1.43 nop	-87,9
G27	119.33 bc	8.17 ijk	-93,2	11.67 i-o	4.00 klm	-65,7	9.39 c-f	1.43 nop	-84,8
G28	109.00 b-e	7.33 ijk	-93,3	14.00 d-k	4.00 klm	-71,4	9.99 b-f	1.41 nop	-85,9
G29	113.67 bcd	14.00 g-k	-87,7	14.67 c-j	5.00 h-m	-65,9	9.62 c-f	1.94 m-p	-79,8
G30	10.00 q	5.33 k	-46,7	11.00 j-q	4.67 i-m	-57,6	6.52 j-m	2.18 k-p	-66,6
G31	128.00 b	26.33 d-g	-79,4	16.33 c-g	5.33 g-l	-67,4	10.93 abc	6.40 def	-41,5
G32	163.00 a	27.67 c-f	-83,0	18.33 bc	4.67 i-m	-74,5	8.96 d-g	4.43 ghi	-50,6
G33	117.00 bcd	12.67 h-k	-89,2	10.67 j-q	3.00 m	-71,9	8.17 f-k	3.01 i-n	-63,2
G36	42.80 k-p	5.33 k	-87,6	13.83 d-k	3.67 lm	-73,5	8.38 f-i	2.47 k-o	-70,5
G38	189.00 a	8.00 ijk	-95,8	15.67 c-i	4.67 i-m	-70,2	9.08d-g	1.72 m-p	-81,1
G39	24.60 opq	3.10 k	-87,4	16.67 c-f	5.50 f-l	-67,0	5.88 l-o	1.47 nop	-75,0
G40	81.60 e-j	3.00 k	-96,3	18.33 bc	5.33 g-l	-70,9	6.75 i-m	1.41 nop	-79,1
G41	27.60 n-q	3.67 k	-86,7	12.00 h-o	5.00 h-m	-58,3	4.45 no	0.78 p	-82,5
G42	34.50 l-q	3.33 k	-90,4	17.67 bcd	6.67 c-i	-62,3	6.51 j-m	1.47 nop	-77,4
G43	35.67 l-q	3.33 k	-90,7	21.00 b	6.00 e-k	-71,4	5.45 mno	2.02 k-p	-62,9
G44	60.00 h-m	5.33 k	-91,1	15.67 c-i	7.00 b-h	-55,3	5.48 mno	1.15 op	-79,0
G45	7.33 q	2.50 k	-65,9	10.33 k-q	5.00 h-m	-51,6	5.40 mno	1.08 op	-80,0
G46	8.33 q	4.33 k	-48,0	7.00 q	3.67 lm	-47,6	5.28 mno	1.99 l-p	-62,3
G47	53.60 j-o	5.50 k	-89,7	9.00 n-q	5.00 h-m	-44,4	6.05 l-o	3.63 h-l	-40,0
G48	29.70 m-q	7.33 ijk	-75,3	11.00 j-q	6.33 d-j	-42,5	5.98 l-o	4.14 hij	-30,8
G49	15.33 p-q	6.00 jk	-60,9	8.67 opq	4.00 klm	-53,9	5.95 l-o	1.73 m-p	-70,9
G50	29.17 m-q	7.33 ijk	-74,9	7.33 pq	4.00 klm	-45,4	5.80 l-o	2.42 k-p	-58,3
G51	8.33 q	4.00 k	-52,0	10.00 k-q	3.67 lm	-63,3	4.33 o	1.01 op	-76,7
cv	0.27	0.53	-	0.19	0.21	-	0.14	0.28	-

[a]Different letters in the same column indicate significant differences P<0.05.

Table 3. Changes in some plant vegetative growth parameters and scale scores of Cucurbit genotypes based on the leaf damage under salt stress (16 dS m^{-1}).

Genotype	Leaf area (cm^2)			Shoot dry weight (g)			Root dry weight (g)			SCORE 16 dS m^{-1}
	0 dS m^{-1}	16 dS m^{-1}	Diff. (%)	0 dS m^{-1}	16 dS m^{-1}	Diff. (%)	0 dS m^{-1}	16 dS m^{-1}	Diff. (%)	
G1	67.50 n-s[a]	19.10 l-p	-71,7	4.40 m-s	2.26 e-m	-48,6	1.70 a-e	0.53 fg	-68,8	4.35 bc
G2	67.90 n-s	29.03 g-p	-57,3	7.40 j-m	2.87 c-j	-61,2	1.38 d-j	0.87 cde	-37,0	4.00 cde
G3	87.00 m-q	27.47 g-p	-68,4	5.90 l-r	2.80 c-k	-52,5	1.30 d-k	0.90 cde	-30,8	3.75 de
G4	88.00 m-q	38.40 f-n	-56,4	7.20 k-n	2.50 d-l	-65,3	2.40 abc	0.37 gh	-84,6	2.50 e
G5	78.10 m-r	37.35 f-n	-52,2	9.60 h-k	4.15 a-d	-56,8	2.60 a	0.80 c-f	-69,2	4.50 abc
G6	78.70 m-r	23.80 i-p	-69,8	5.90 l-r	3.56 b-f	-39,7	1.20 d-m	0.60 efg	-50,0	4.25 bcd
G7	80.83 m-r	54.20 ef	-33,0	5.80 l-r	3.90 a-e	-32,8	2.50 ab	1.47 a	-41,2	4.00 cde
G8	96.30 m-p	23.17 f-o	-75,9	8.00 jkl	4.20 abc	-47,5	1.23 d-m	1.10 hi	-10,6	4.25 bcd
G9	123.00 j-n	46.60 e-j	-62,1	8.60 i-l	4.70 ab	-45,4	1.60 b-f	0.93 bcd	-41,9	4.00 cde
G10	112.60 k-o	20.32 k-p	-82,0	7.10 k-o	3.23 b-h	-54,5	1.00 d-o	0.50 fg	-50,0	4.75 ab
G11	57.90 o-s	21.76 j-p	-62,4	6.30 l-p	4.70 ab	-25,4	1.20 d-m	0.70 def	-41,7	4.25 bcd
G12	332.40 a-d	80.22 bcd	-75,9	6.30 l-p	4.63 ab	-26,5	1.90 a-d	0.63 d-g	-66,8	4.00 cde
G13	166.70 h-k	117.20 a	-29,7	11.70 f-i	4.00 a-d	-65,8	1.00 d-o	0.63 d-g	-37,0	2.00 f
G14	97.60 m-p	54.62 ef	-44,0	9.60 h-k	4.63 ab	-51,8	2.50 ab	1.03 bc	-58,8	3.75 de
G15	99.60 m-p	44.11 e-k	-55,7	8.10 jkl	5.50 a	-32,1	1.70 a-e	0.80 c-f	-52,9	3.75 de
G16	122.50 j-n	8.70 op	-92,9	6.16 l-q	0.33 opq	-94,6	0.79 e-o	0.06 i	-92,4	5.00 a
G17	158.33 h-l	14.63 m-p	-90,8	8.45 jkl	0.63 m-q	-92,5	0.77 e-o	0.11 hi	-85,7	5.00 a
G18	74.77 n-s	10.93 op	-85,4	2.22 stu	0.25 pq	-88,7	0.51 h-o	0.08 hi	-84,3	5.00 a
G19	257.03 ef	49.91 e-h	-80,6	17.27 abc	2.07 f-n	-88,0	1.15 d-m	0.16 hi	-86,1	5.00 a
G20	236.93 fg	18.13 l-p	-92,4	10.47 g-j	0.80 m-q	-92,4	0.60 g-o	0.07 hi	-88,3	5.00 a
G21	376.83 a	52.00 efg	-86,2	15.92 bcd	1.85 g-p	-88,4	1.06 d-n	0.14 hi	-86,8	5.00 a
G22	37.20 qrs	10.92 op	-70,7	2.85 r-u	0.08 q	-97,2	0.37 k-o	0.08 hi	-78,4	5.00 a
G23	314.20 b-e	82.21 bc	-73,8	13.26 d-g	1.36 j-q	-89,7	1.04 d-o	0.08 hi	-92,3	5.00 a
G24	294.50 de	37.40 f-n	-87,3	15.44 b-e	1.37 j-q	-91,1	1.38 d-j	0.07 hi	-94,9	5.00 a
G25	308.37 b-e	39.13 f-m	-87,3	18.59 ab	1.62 h-q	-91,3	1.48 c-g	0.15 hi	-89,9	5.00 a

Continuing table 3

Genotype	Leaf area (cm^2)			Shoot dry weight (g)			Root dry weight (g)			SCORE 16 dS m^{-1}
	0 dS m^{-1}	16 dS m^{-1}	Diff. (%)	0 dS m^{-1}	16 dS m^{-1}	Diff. (%)	0 dS m^{-1}	16 dS m^{-1}	Diff. (%)	
G26	353.40 abc	56.97 def	-83,9	15.06 cde	2.10 f-n	-86,1	1.38 d-j	0.11 hi	-92,0	5.00 a
G27	298.63 cde	51.82 efg	-82,7	16.06 bcd	2.00 f-n	-87,6	1.27 d-l	0.16 hi	-87,4	5.00 a
G28	235.17 fg	38.73 f-n	-83,5	12.39 e-h	1.71 g-q	-86,2	1.62 b-f	0.20 hi	-87,7	5.00 a
G29	362.46 ab	66.93 cde	-81,5	19.46 a	3.31 b-g	-83,0	1.40 d-i	0.20 hi	-85,7	4.00 cde
G30	61.87 o-s	8.62 op	-86,1	2.83 r-u	0.72 m-q	-74,6	0.60 g-o	0.12 hi	-80,0	4.00 cde
G31	379.60 a	93.77 ab	-75,3	17.30 abc	4.01 a-d	-76,8	1.30 d-k	0.16 hi	-87,7	4.00 cde
G32	359.60 ab	97.28 ab	-73,0	13.95 def	3.10 b-i	-77,8	1.13 d-m	0.13 hi	-88,5	5.00 a
G33	210.07 fgh	39.73 f-l	-81,1	7.25 k-n	1.55 i-q	-78,6	0.94 d-o	0.07 hi	-92,6	-
G36	232.50 fg	46.67 e-i	-79,9	7.80 jkl	1.52 i-q	-80,5	0.67 f-o	0.09 hi	-86,6	5.00 a
G38	262.53 ef	45.87 e-j	-82,5	14.41 c-f	1.99 f-o	-86,2	1.43 d-h	0.10 hi	-93,0	5.00 a
G39	88.17 m-q	13.93 nop	-84,2	2.52 stu	0.56 n-q	-77,8	0.29 mno	0.01 i	-96,6	5.00 a
G40	188.40 ghi	15.88 l-p	-91,6	8.49 jkl	1.17 k-q	-86,2	0.55 g-o	0.07 hi	-87,3	4.00 cde
G41	51.10 p-s	9.13 op	-82,1	1.10 tu	0.47 n-q	-57,3	0.09 o	0.03 i	-66,7	5.00 a
G42	57.52 o-s	15.00 l-p	-73,9	3.59 p-u	0.91 l-q	-74,7	0.42 j-o	0.05 i	-88,1	5.00 a
G43	132.27 i-m	11.35 op	-91,4	4.28 m-s	1.02 l-q	-76,2	0.32 l-o	0.05 i	-84,4	5.00 a
G44	120.20 j-n	18.07 l-p	-85,0	4.13 n-t	0.98 l-q	-76,3	0.25 mno	0.04 i	-84,0	5.00 a
G45	18.23 s	5.75 p	-68,5	0.58 u	0.61 m-q	5,2	0.09 o	0.01 i	-88,9	5.00 a
G46	35.60 qrs	11.75 op	-67,0	1.10 tu	0.52 n-q	-52,7	0.13 no	0.03 i	-76,9	5.00 a
G47	172.40 hij	25.40 h-p	-85,3	5.80 l-r	1.15 k-q	-80,2	0.29 mno	0.05 i	-82,8	5.00 a
G48	72.20 n-s	20.37 k-p	-71,8	2.35 stu	1.23 j-q	-47,7	0.27 mno	0.08 hi	-70,4	4.50 abc
G49	108.07 l-p	19.45 k-p	-82,0	3.04 q-u	1.21 j-q	-60,2	0.24 mno	0.05 i	-79,2	5.00 a
G50	173.80 hij	22.75 i-p	-86,9	4.00 o-t	1.30 j-q	-67,5	0.46 i-o	0.13 hi	-71,7	5.00 a
G51	25.77 rs	7.92 op	-69,3	1.30 stu	0.61 m-q	-53,1	0.13 no	0.05 i	-61,5	5.00 a
cv	0.22	0.43	-	0.24	0.49	-	0.58	0.24	-	0.08

[a]Different letters in the same column indicate significant differences P<0.05.

Figure 1. Sodium accumulation in leaves of different Cucurbit genotypes grown in salt treatment (16 dS m^{-1}) and control medium.

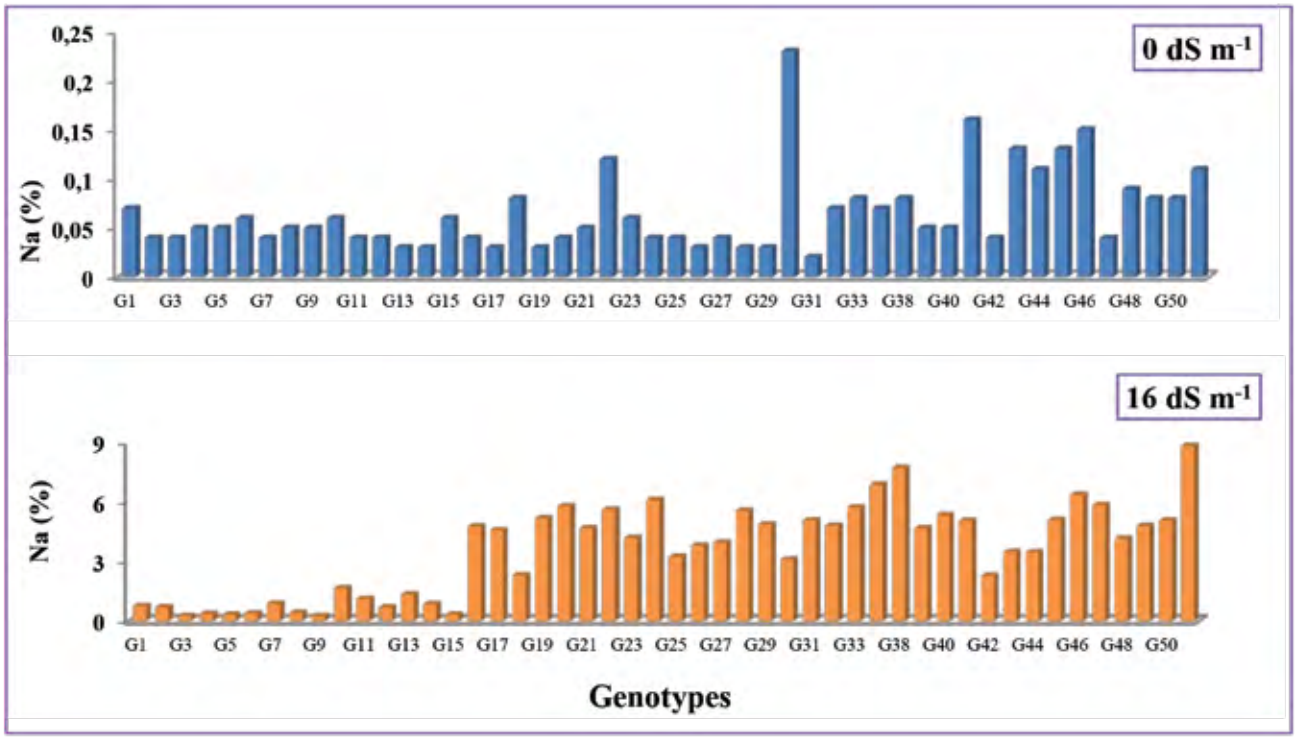


Figure 2. Potassium accumulation in leaves of different Cucurbit genotypes grown in salt treatment (16 dS m^{-1}) and control medium.

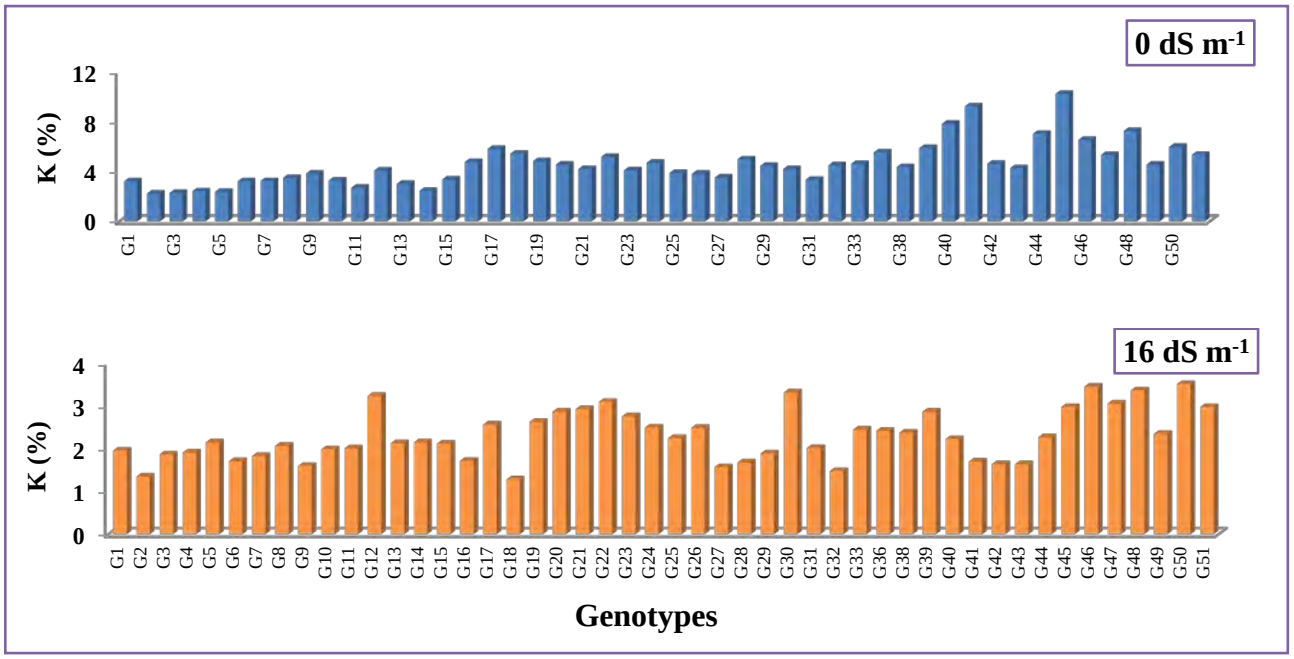


Figure 3. Calcium accumulation in leaves of different Cucurbit genotypes grown in salt treatment (16 dS m^{-1}) and control medium.

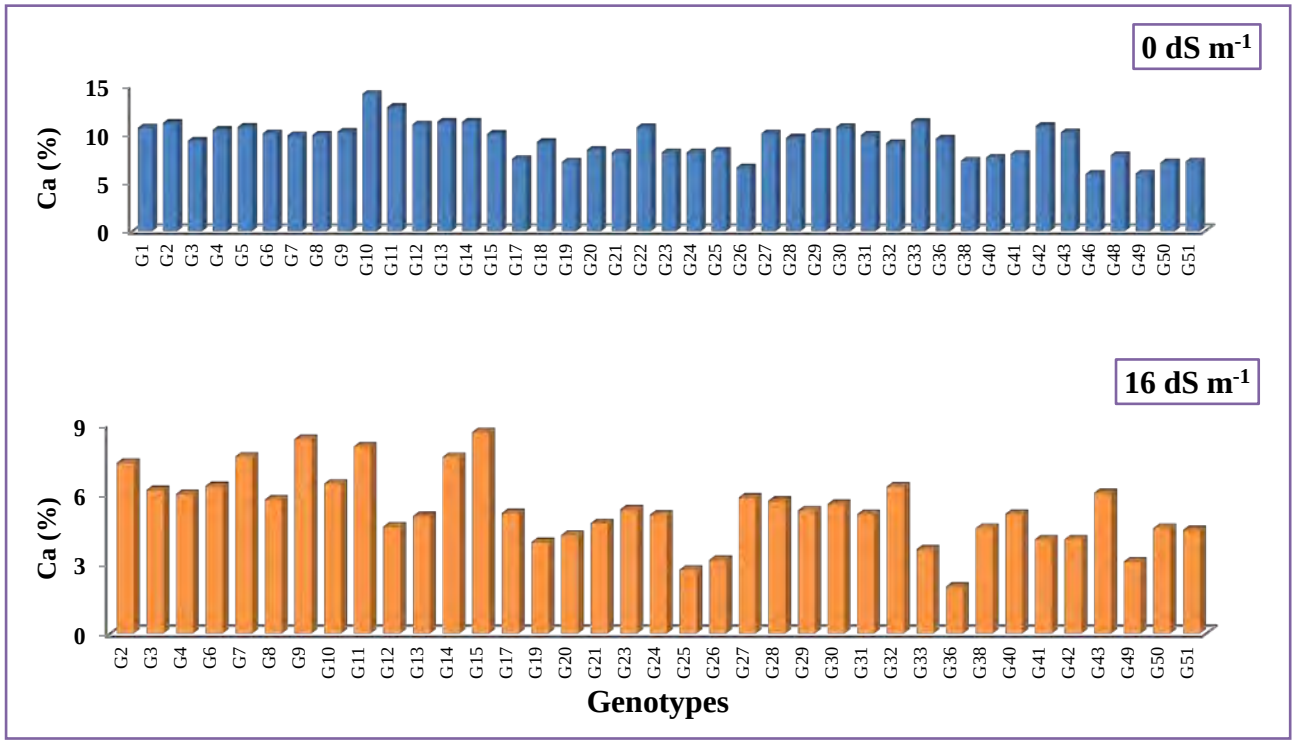


Figure 4. K/Na ion contents of different Cucurbit genotypes grown in salt treatment (16 dS m^{-1}) and control medium.

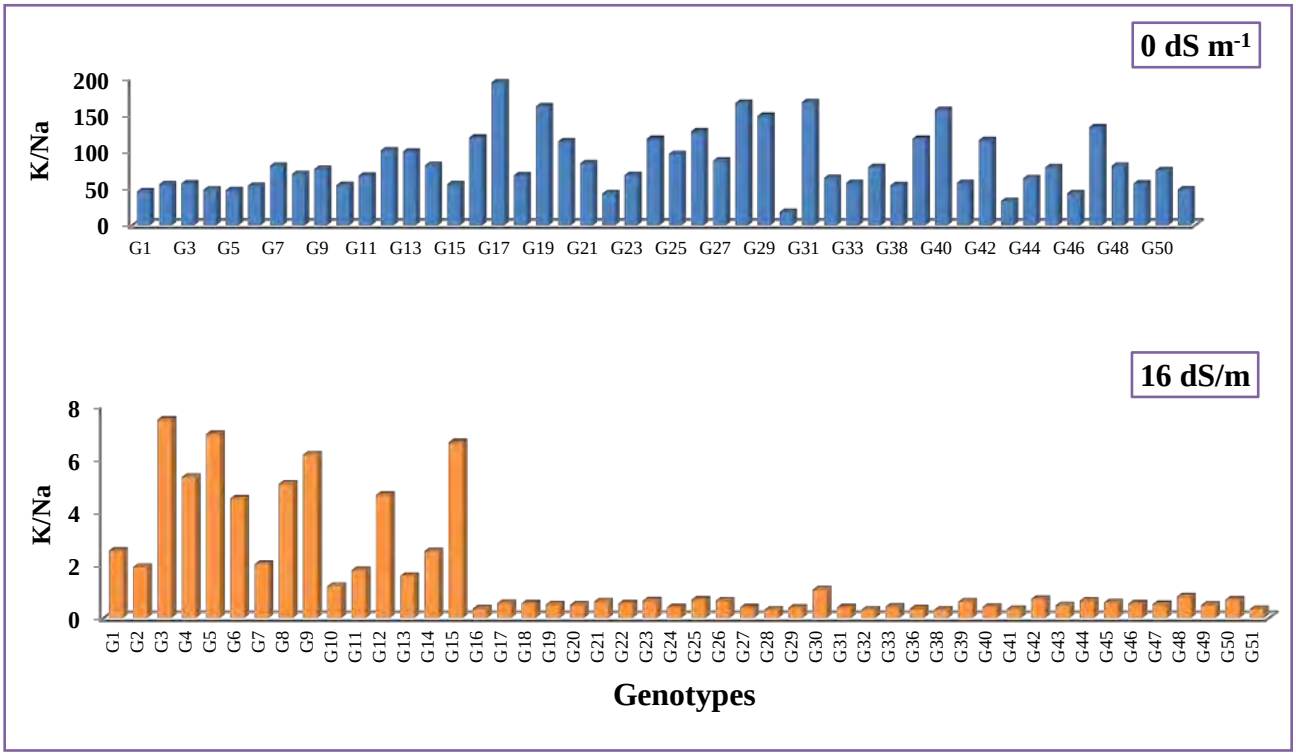
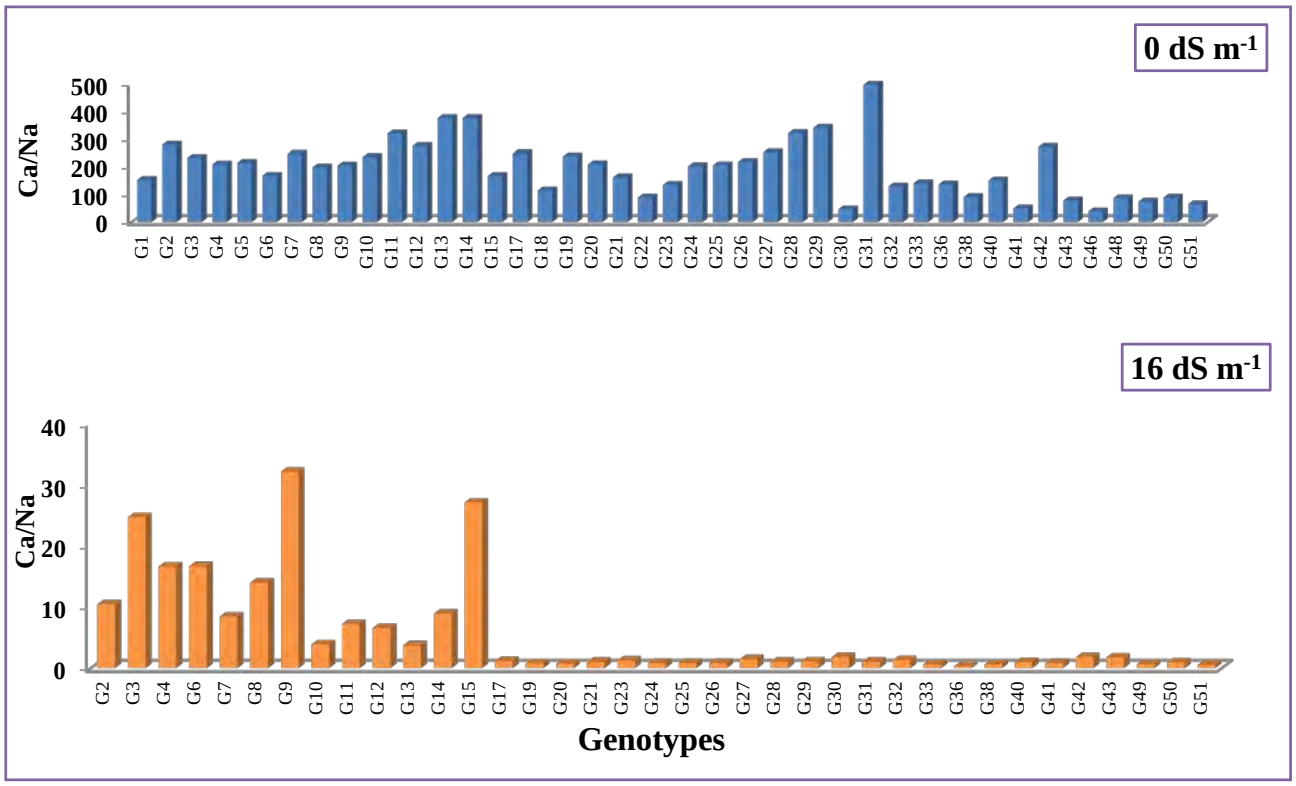


Figure 5. Ca/Na ion contents of different Cucurbita genotypes grown in salt treatment (16 dS m^{-1}) and control medium.



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Variability for Agro-Morphological Traits of Maize (*Zea mays* L.) Inbred Lines Differing in Drought Tolerance

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ABSTRACT

Drought tolerant genotypes have high yield in optimal conditions and lower, but stable yield in dry environments. Gene bank collection (6,000 accessions) of Maize Research Institute was tested under controlled drought in Egypt, and in temperate climate. The mini-core collection of 15 inbreds and 26 populations was created. Inbreds together with lines B73, A632, Mo17 and few commercial inbreds with different tolerance to drought, were evaluated for agro-morphological traits (plant and ear height, total number of leaves, number of leaves above ear, ear leaf length and width), grain yield, number of rows per ear and number of kernels per row, under optimal and increased density in the field in 2014. Since optimal precipitation for maize growing in Serbia is 425 mm, total precipitations of 873.2 mm along with the average temperature of 18.8˚C were exceptionally good for maize production. According to Principal Component Analysis, traits that contributed to the differentiation and were in common for both densities were: number of kernels per row, grain yield and leaf width. Obtained results indicated that inbreds T4 and T8 performed the highest stability, together with commercial T1 and T2 lines, in both experimental conditions. Cluster analysis based on grain yield and morphological traits, grouped them together with the other drought tolerant lines, apart of B73 and lines that showed sensitivity to drought in previous studies. Higher density conditions, simulating mild stress, contributed to more accurate separation of lines from mini-core collection, which could be used as a source for drought tolerance in breeding programs.

Keywords: drought, inbred lines, gene bank, maize, grain yield.

Introduction

The major implications for global food supply are addressed to drought, due to the expected gradual climate change effects over the next century, and the variation in climatic extremes in the short term that it is expected to bring. Increased temperature is a more predictable outcome than changes in rainfall patterns accompanying climate change (Edmeades, 2013). Moreover, as a general notion, major maize producing areas will be subjected to an evolving array of maize diseases and pests that are new to those areas.

In the regions relying on in-season rainfall, there is considerable inter-annual variation in rainfall total and distribution (Löffler *et al.,* 2005). In some years, yield can be significantly reduced by transient water limitations of varying timing, duration, and severity. Many of these water limitations have minor to moderate impact on yield. However, widespread and prolonged drought that substantially reduces grain yield over a wide area can occur in some years, as in 2012 being the most recent occurrence (Boyer *et al.,* 2013).

Maize (*Zea mays* L.) is one of the most important cereal crops with the largest annual global production (Golbashy *et al.,* 2010). However, most of the 160 M ha of production area is highly affected by drought. The EU countries produce around 12% of global maize, and high air temperatures and water deficit in 2012 reduced their yields by an average of 12.5% (MARS, 2012). In Serbia, maize is the most important crop grown mainly without irrigation, which seriously affected genetic potential for yield (Videnovic *et al.,* 2013), and its yield in dry 2012 has been reduced by an average of 48%.

Breeding progress relies on considerable genetic variability for the trait of interest (e.g. grain yield under drought stress), high selection intensity through screening a large number of genotypes and high broad-sense heritability for the trait of interest (Grzesiak, 2001). Thus, the ability to develop high yielding and stable cultivars is an ultimate goal in most breeding programs. The consistent performance of a genotype, both with high or low yield across different environments is considered as yield stability (Epinat-Le Signor *et al.,* 2001). An ideal maize genotype should have a high mean yield combined with a low degree of fluctuation under different environments (Annicchiarico, 2002). Since drought stress influences the reduction of growth, development and production of plants (Mohammadai *et al.,* 2012), one of the most important goals for maize breeders has been to enhance the stability of performance of maize when exposed to stresses (Campos *et al.,* 2006).

Maize Research Institute "Zemun Polje" gene bank maintains 5,806 accessions and is among the ten largest in the world (FAOSTAT, 2010), thus offering the great opportunities for different breeding purposes. After two-year of screening for drought tolerance in Egypt under managed stress environment (MSE) conditions and further testing in the temperate climate regions of Macedonia and Serbia, a mini-core collection of 41 accessions (15 maize inbred lines, 13 local and 13 introduced landraces) was established (Vancetovic *et al.,* 2010; Babic *et al.,* 2011).

Understanding the environmental and agronomic responses of maize varieties is fundamental to improve the efficiency of maize production. Accordingly, we evaluated a set of 15 maize inbred lines from drought tolerant mini-core collection, together with lines B73, A632, Mo17 and few commercial inbreds with different tolerance to drought, under optimal and increased density in the field. By measuring agro-morphological traits of importance, the aim of this study was to distinguish genotypes from drought tolerant mini-*core* collection with a high mean yield combined with a low degree of fluctuation under different environmental conditions applied.

Materials and Methods

Public maize inbred lines B73, A632, Mo17, three drought susceptible (S1, S2 and S3) and two drought tolerant (T1 and T2) commercial inbreds, along with a set of 15 maize inbred lines from Maize Research Institute "Zemun Polje" drought tolerant mini-core collection (from T3 to T17) were evaluated in the present study.

The experiment was carried out in 2014 in Zemun Polje, Serbia (44°52´N, 20°19´E, 81 m asl), in two plant densities. The soil was slightly calcareous chernozem with 47% clay and received the usual compound of mineral fertilizer. Chosen inbreds were tested in two-replicate trial, set up according to Randomised Complete Block Design. Plants of each genotype were sown in a single row plot per replica, with 10 hills per row and spaced 0.75 m apart. Spacing between hills were 20 cm (i.e. D-20) and 40 cm (i.e. D-40), respectively. Plots were overplanted and thinned to two plants per hill after seedling establishment. Morphological traits, such as - plant height (PH), total number of leaves (TNL), number of leaves above upper most ear (LAE), ear height (EH), leaf length (LL), leaf width (LW) and grain yield (Y) were recorded for each entry in both replicates, on ten representative plants per maize inbred. Grain yield was calculated per plant, after manual harvesting and drying to 14% of moisture content. Yield components: number of kernel rows per ear (NKR) and number of kernels per row (NKR) were recorded on ten randomly chosen ears.

Data matrix was constructed according to mean values for seven agro-morphological traits observed and their standard deviations (SD). Cluster analysis was conducted using square Euclidean distance and complete linkage method. Principal Component Analysis (PCA) was performed based on the phenotypic correlation matrix of the adjusted means of the inbred lines for the nine agro-morphological traits using SPSS 16.0 (http://spss-for-windows-evaluation-version.software.informer.com/). The matrix of distances between maize inbreds was calculated upon the standardized principal components with eigenvalue higher than one. Traits with a correlation > 0.7 were considered as significant for that component. Correlation analysis between the traits observed was performed using Pearson's correlation coefficient.

Results and Discussion

Grain maize producers in Europe experienced an excellent season in 2014, that led to record yields for the EU countries as a whole (MARS, 2014). In Serbia, maize grain yield was around 25% above the five-year average. Rainfall accumulation of 863.2 mm, exceeded by far the optimal sum of precipitation (e.g. 425 mm), estimated for maize vegetative period (Vasic and Kerecki, 1988). Near optimal ammount of rainfall during June, laid the foundation for a good season. Moreover, the rains of July (150 mm above the optimum level) were especially beneficial to the growth of maize, reflected in remarkably vigorous canopy expansion. Ample soil moisture levels sustained the flowering phase and the subsequent early grain-filling period with a very positive effect on yield formation. Fewer hot days (Tmax > 30°C) were recorded in June, July and August (7, 8 and 7, respectively). Consequently, no drought or extraordinary hot spells compromised the pollination of maize. In August, water supply was at near optimal levels during the grainfilling stages. However, abundant rainfall and overly wet soil conditions in September (around 80 mm above the optimum level), hampered and caused significant delays to the harvest, which mainly increased the drying costs, but did not cause considerable yield losses. Thus, extremely high rainfall accumulation in 2014, allowed us to evaluate yield stability among maize inbred lines previously chosen as drought tolerant.

Yield and yield stability across diverse environments and multiple years (i.e. weather conditions) are some of the most important selection targets for plant breeding (Moose and Mumm, 2008). Genotypes with yield stability tend to have higher stress tolerance (Tollenaar and Lee, 2002) and demonstrate greater resource use efficiency (Ipsilandis and Vafias, 2005), allowing them to reach more of their total yield potential as the maximum yield achieved under stress-free growing conditions and nonlimiting resources (Fasoula and Fasoula, 2002).

Plants grown in the same field compete for basic requirements for growth (i.e. sunlight, moisture, and nutrients from the soil), thus, increase of plant population would, to a certain extent, induce stress on the genotypes. Considering that, two plant densities were applied in our experiment: D-40, simulating stress-free growing condition and nonlimiting resources, and D-20, simulating mild stress-induced conditions. Average values for agro-morphological traits observed were presented in Table 1 and Table 2.

Effect of D-20 applied resulted in the reduction of all examined traits. Reduction for morphological traits ranged from 10.2% for TNL to 12.4% for EH. Compared to D-40 growing conditions, average yield reduction was 30.3%, being the lowest in inbreds from drought tolerant mini-*core* collection (i.e. 29.4% reduction), which was in accordance with the results of Andjelkovic *et al.,* (2014). There is substantial genetic variation for plant density tolerance in maize (Sarlangue *et al.,* 2007). Some genotypes yield more as plant density is increased, as was the case for drought tolerant inbred T3, exhibiting 1.3% of yield increase under D-20 (Hashemi *et al.,* 2005; Grassini *et al.,* 2011). Reduction for yield components were 3.7 % for NRE and 11.5% for NKR, respectively.

Dendrogram based on morphological traits and grain yield obtained under D-20 (Figure 1), can be divided into three main clusters (A, B and C). Two inbreds (T15 and T17) from drought tolerant mini-core collection were assigned to cluster A, characterized with the highest values of morphological traits observed. Also, the inbred T15 obtained relatively high grain yield under D-20 conditions. The inbreds T8 and T11, both assigned to cluster B, achieved the highest grain yield compared to all the genotypes from drought tolerant mini-core collection (45.6 g/plant and 49.1 g/plant, respectively), even in compare to public lines B73 and Mo17. The lowest values for the traits observed characterized inbred lines from the cluster C.

Under D-40 plant density, similar distribution of genotypes was observed (Figure 2). This dendrogram can be divided into two main clusters (A and B). Although there was grain yield reduction for drought tolerant inbreds T4, T8 and T11 under D-20 (i.e. 25.5%, 12.7% and 11.2%, respectively), it is important to notice that those inbreds ranged even better compare to grain yield of the rest of inbreds, achieved under higher plant density.

Breeding programs are based on selection for several traits simultaneously and, therefore, knowledge on the genetic association between them is necessary. Correlations detect the strength of relationships between grain yield and the other examined traits. In this experiment, correlation analyses between grain yield obtained under D-20 conditions and morphological traits (Table 3) have shown significant and positive correlations ($P \leq 0.05$ for EH, $P \leq 0.01$ for LW, and $P \leq 0.001$ for PH and LL, respectively). Similar ternd was observed under D-40.

This was in line with study of Rahman *et al.,* (2015). Also, grain yield was in highly significant and positive correlations with ear traits (e.g. NRE and NKR), which is usual association under favorable conditions (Menkir *et al.,* 2009).

In our experiment, PCA was performed for morphological traits, grain yield and yield components in both densities. Under D-20 conditions, PCA revealed that PH, LW, grain yield and NKR contributed to the first axis (PCA1), which explained 59.707% of the total variability. The second axis (PCA2) which explained 16.091% of the variation was defined with TNL, LAE and NRE (Figure 3). Under D-40 conditions, PCA revealed that the majority of the traits observed contributed to the first axis (PCA1), which explained 58.648% of the total variability among the evaluated maize inbred lines. The second axis (PCA2) which explained 15.558% of the variation was defined only with NRE (Figure 4). PCA helps to identify the traits with the highest variability as well as those ones that characterize the distinctness among selected genotypes. According to PCA, traits that contributed to the differentiation and were in common for both densities were: LW, NKR and grain yield.

Conclusion

Ideal genotypes could be considered those that have a large PCA1 score (high yielding ability) and small or absolute PCA2 score (high stability). Higher density conditions, simulating mild stress, contributed to more accurate separation of evaluated maize inbred lines differing in drought tolerance. Since yield stability reflects to higher stress tolerance and greater resource use efficiency, it can be observed that the inbred line T8 from drought tolerant mini-*core* collection was the closest to the ideal genotype, followed by inbred T4, also from drought tolerant mini-core collection together with commercial T1 and T2 lines. These inbreds could be used as a source for drought tolerance in breeding programs.

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Table 1. Evaluated agro-morphological traits in maize inbred lines differing in drought tolerance.

Inbred line	PH (cm)	EH (cm)	TNL	LAE	LL (cm)	LW (cm)	Y (g/plant)	NRE	NKR
B73	142.5	67.3	17.7	5.8	69.6	8.0	31.1	17.0	21.5
A632	114.9	42.6	16.6	6.1	67.6	6.9	45.0	14.0	21.0
Mo17	121.3	50.6	16.6	4.8	58.6	8.6	30.6	10.0	21.5
S1	144.2	66.3	18.3	6.1	66.6	9.0	54.0	16.0	27.0
S2	124.5	62.5	16.5	5.4	73.7	8.9	43.6	14.0	32.5
S3	127.3	55.8	16.3	5.1	71.6	9.3	55.2	14.0	21.0
T1	119.7	39.3	16.1	5.8	59.1	6.9	38.8	11.0	23.5
T2	138.6	53.3	16.7	5.6	71.7	8.4	70.1	13.0	31.5
T3	91.4	23.8	13.3	5.1	54.6	5.8	30.0	12.0	19.0
T4	110.0	40.8	15.0	5.0	61.5	8.2	39.6	14.0	24.5
T5	102.8	42.6	14.6	4.7	59.2	6.6	23.6	14.0	24.5
T6	85.5	29.3	12.1	3.9	44.8	6.8	18.7	13.0	18.0
T7	74.3	37.8	15.0	4.5	49.4	7.3	16.1	10.0	18.5
T8	111.9	41.0	17.1	5.6	58.0	8.9	45.6	13.0	27.0
T9	103.3	50.2	13.2	4.9	60.2	8.6	42.3	15.0	18.5
T10	92.8	42.4	15.2	5.3	51.7	7.3	19.2	10.0	18.5
T11	110.5	54.0	14.7	5.1	59.6	7.0	49.1	16.0	23.0
T12	102.0	38.3	13.1	5.0	55.7	7.2	37.7	14.0	24.0
T13	98.5	41.8	13.1	4.0	55.9	7.9	40.3	12.0	26.0
T14	91.3	36.8	14.5	5.2	49.3	6.9	35.8	13.0	21.0
T15	147.4	68.3	17.3	6.0	71.3	8.2	49.0	16.0	27.0
T16	97.5	32.5	14.3	4.9	49.8	5.6	29.1	14.0	21.5
T17	136.3	54.8	18.4	6.9	73.9	8.1	31.8	18.0	28.5

The results present mean values of two replications for D-20 plant density applied (20 cm between hills in the row). S - drought susceptible inbred line; T - drought tolerant inbred line.

Table 2. Evaluated agro-morphological traits in maize inbred lines differing in drought tolerance.

Inbred line	PH (cm)	EH (cm)	TNL	LAE	LL (cm)	LW (cm)	Y (g/plant)	NRE	NKR
B73	151.1	74.4	18.9	6.5	73.1	8.7	47.7	17.0	25.0
A632	120.5	49.8	17.6	6.9	71.0	7.1	56.7	13.0	24.5
Mo17	141.8	67.8	16.7	5.1	58.9	9.1	21.5	10.0	27.0
S1	148.3	80.0	18.9	6.2	70.3	10.0	92.8	16.0	30.0
S2	131.8	65.8	18.1	5.7	76.2	9.5	67.3	14.0	32.5
S3	135.0	57.5	17.7	6.2	74.9	10.2	77.4	15.5	25.0
T1	132.9	42.0	17.9	6.8	63.4	8.0	69.0	12.0	31.0
T2	145.5	61.0	18.0	6.2	74.7	9.6	88.2	14.0	30.0
T3	100.9	27.5	14.4	5.5	57.5	6.1	29.6	13.0	22.0
T4	114.5	41.6	16.3	6.0	67.3	8.9	53.2	14.0	29.0
T5	110.5	48.8	15.2	5.6	67.8	7.0	37.2	14.0	25.5
T6	89.6	32.3	13.2	4.3	46.6	7.0	33.2	14.0	19.5
T7	81.3	43.3	15.8	5.3	51.9	8.0	29.9	12.0	20.5
T8	119.0	49.3	17.2	6.2	61.5	10.5	52.3	14.0	29.0
T9	116.3	60.5	14.6	5.5	66.2	9.1	61.6	15.0	21.0
T10	103.3	47.0	16.2	5.9	54.3	7.6	26.1	10.0	21.0
T11	115.8	59.8	15.2	5.2	61.1	7.5	55.2	16.0	25.0
T12	116.0	46.6	13.8	5.0	60.3	7.5	57.3	15.0	23.5
T13	107.5	47.0	13.6	5.1	59.8	8.6	49.1	12.0	26.5
T14	93.8	43.8	15.2	5.7	51.5	7.3	52.3	13.0	26.0
T15	156.6	73.3	17.4	6.9	77.2	9.0	76.3	16.0	29.0
T16	110.5	40.3	15.5	6.4	54.7	6.4	35.8	15.0	22.0
T17	148.5	66.3	19.1	7.3	77.8	8.4	52.3	17.0	32.5

The results present mean values of two replications for D-40 plant density applied (40 cm between hills in the row). S - drought susceptible inbred line; T - drought tolerant inbred line.

Table 3. Phenotypic correlations between agronomic and morphological traits in chosen maize inbred lines under different densities.

	D-20							
	NRE	**NKR**	**PH**	**EH**	**TNL**	**LAE**	**LL**	**LW**
Y	0.354^{ns}	0.598**	0.652***	0.514*	0.399^{ns}	0.389^{ns}	0.650***	0.534**
NRE		0.357^{ns}	0.592**	0.576**	0.363^{ns}	0.520*	0.586**	0.217^{ns}
NKR			0.630**	0.516*	0.524*	0.444*	0.655***	0.469*
	D-40							
	NRE	**NKR**	**PH**	**EH**	**TNL**	**LAE**	**LL**	**LW**
Y	0.510*	0.588**	0.608**	0.527**	0.505*	0.439*	0.677***	0.597**
NRE		0.222^{ns}	0.456*	0.450*	0.297^{ns}	0.348^{ns}	0.545**	0.191^{ns}
NKR			0.691***	0.512*	0.683***	0.537**	0.672***	0.558**

D-20 and D-40 - 20 cm and 40 cm between hills in the row, respectively; *** - significant at the 0.001 probability level; ** - significant at the 0.01 probability level; * - significant at the 0.05 probability level; ^{ns} - non-significant;

Figure 1. Dendrogram of the 23 maize inbred lines differing in drought tolerance constructed using UPGMA cluster analysis of Euclidean distance values obtained by morphological data and grain yield under D-20 plant density.

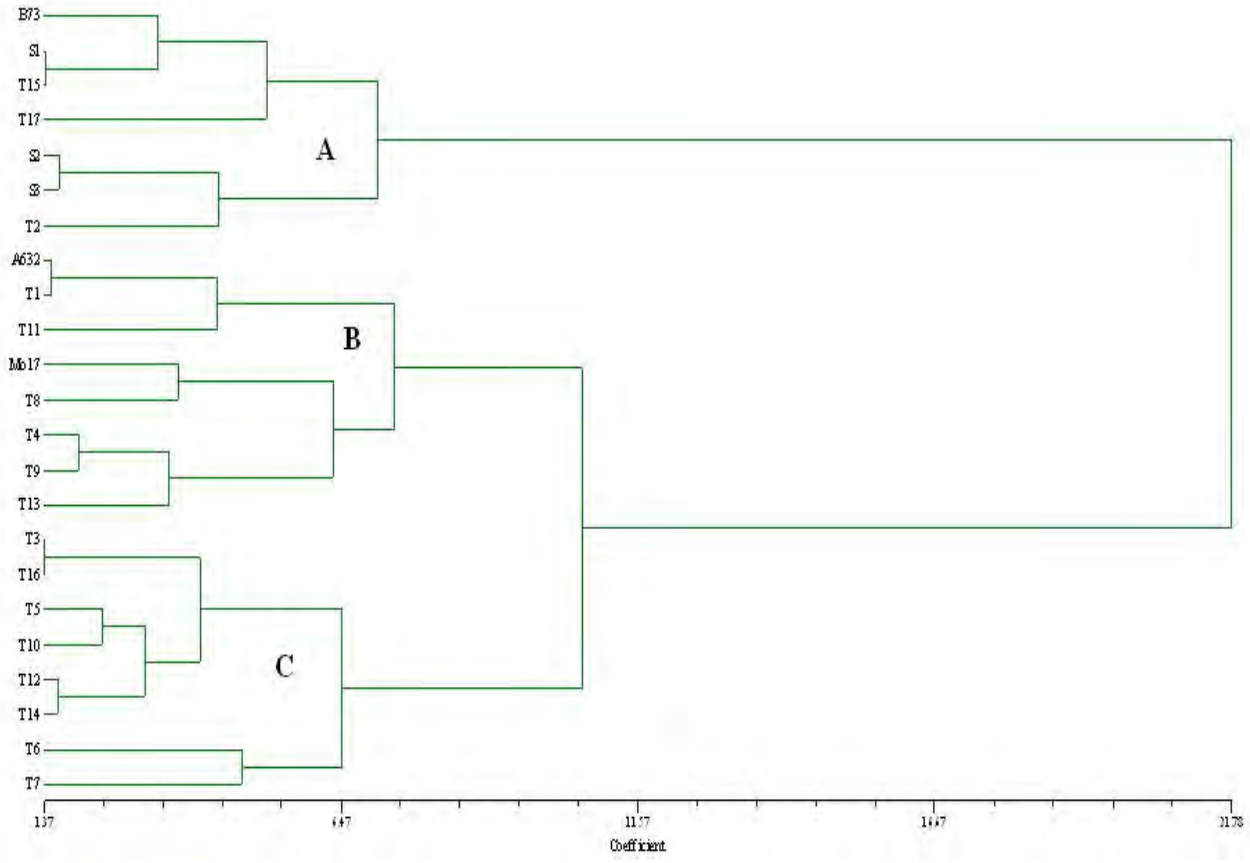
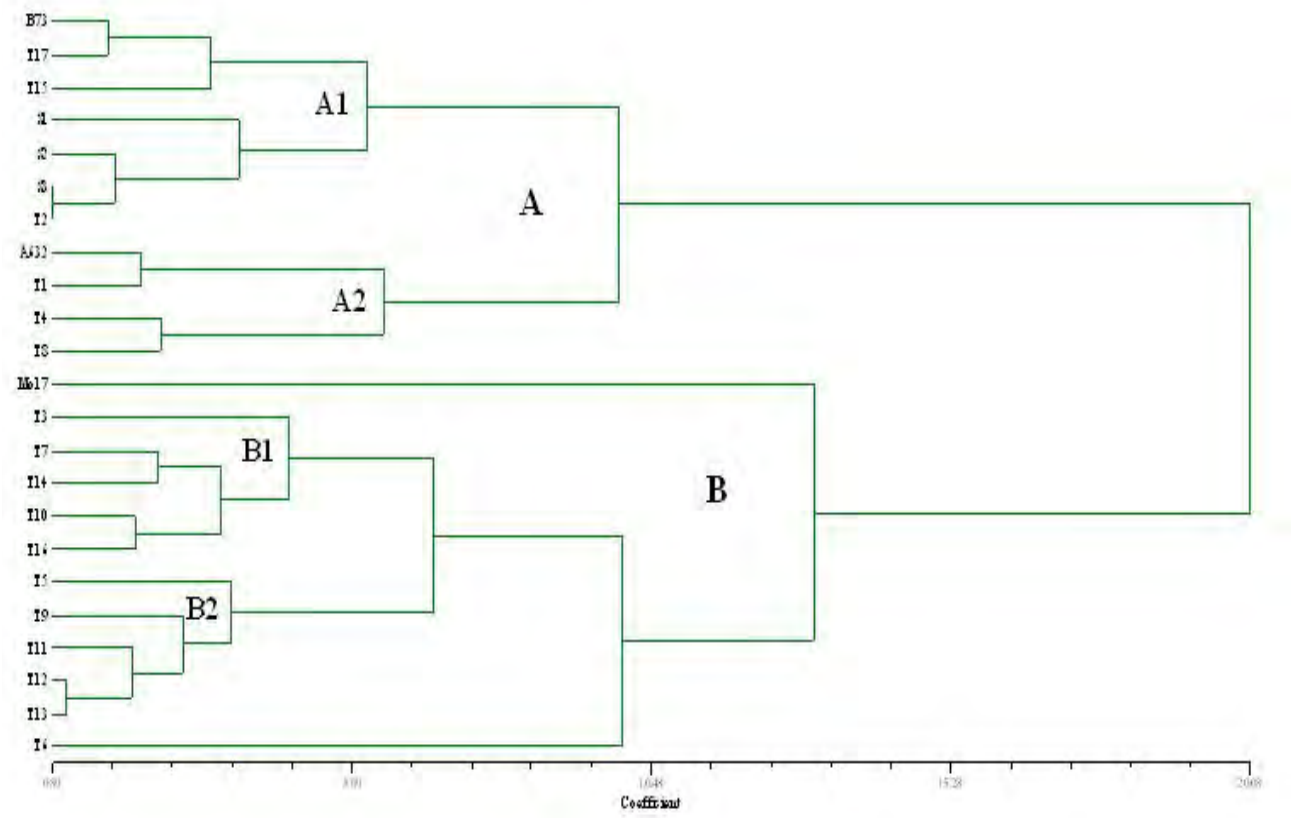


Figure 2. Dendrogram of the 23 maize inbred lines differing in drought tolerance constructed using UPGMA cluster analysis of Euclidean distance values obtained by morphological data and grain yield under D-40 plant density.



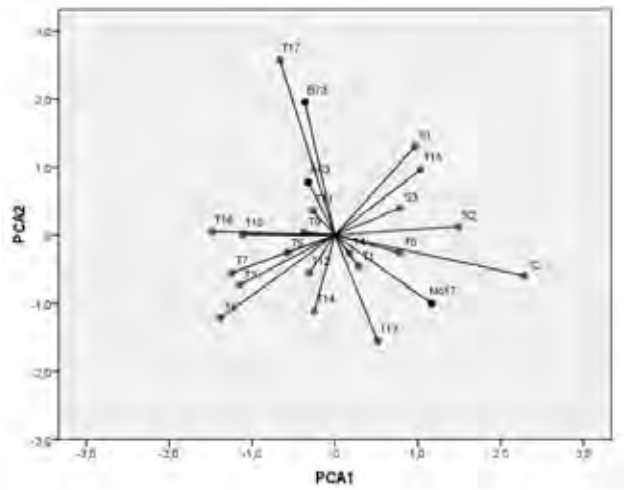


Figure 3. Distribution of the 23 maize inbred lines differing in drought tolerance on the first two principal components PCA1 and PCA2 of the PCA performed for agro-morphological data obtained under D-20 growing conditions.

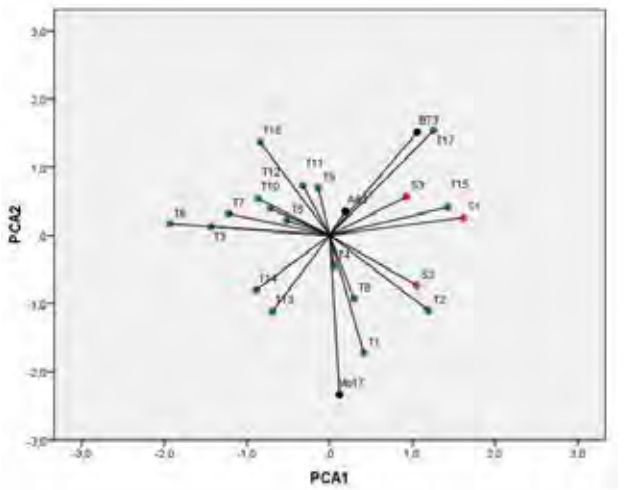


Figure 4. Distribution of the 23 maize inbred lines differing in drought tolerance on the first two principal components PCA1 and PCA2 of the PCA performed for agro-morphological data obtained under D-40 growing conditions.

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Evaluation for Morphological and Biochemical Traits Related to Quality Biomass Production among MS Based Forage Sorghum Hybrids

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ABSTRACT

Livestock is a major component of Indian agriculture. With the increase in livestock population during past few years there is huge gap in demand and supply of green as well as dry fodder, hence forage crops improvement programme needs to be strengthened. Keeping this in view hybrid evaluation experiments were conducted during *kharif* 2013 and 2014 at Research area of forage farm at CCS HAU, Hisar and material was sown in randomized block design to estimate overall performance of hybrids for two successive years. Fifteen MS based hybrids were developed for two successive years and then evaluated for two years for various agronomic traits that affect the biomass production directly or indirectly. Out of these hybrids HH 602, HH 561, HH 529 and HH 619 showed higher green and dry fodder yield for two successive years as compared to best check SSG 59-3 (*GFY: 737.6* q/ha and DFY: 137.7 *q/ha*). As far as their quality is concerned they have low HCN than toxic limit (200 micro g/g) crude protein more than 8% and *in vitro dry matter digestibility* more than 50% in HH 529 and HH 619. Thus, on the basis of this evaluation programme we can say that these hybrids are promising for green fodder productivity. They may also help to fill the gap between demand and supply of green as well as dry fodder for livestock industry.

Keywords: Sorghum, biomass, hybrid, quality and fodder.

Introduction

Sorghum [*Sorghum bicolor* (L.) Moench] is the gifted genera of the tropical regions that provide food, feed, stover (dry straw) and fuel to millions of poor farmer families and their livestock's (Shinde *et al.*, 2015) Single-cut and multi-cut sorghum varieties/ hybrids are also cultivated for green fodder (forage). Sorghum has a wider range of adaptability and is more widely grown. Forage sorghum hybrids are commonly grown in areas where rainfall is insufficient for corn (*Zea mays* (L.) production and may be utilized as silage, greenchop, pasture, dry hay or fodder (Dhalberg *et al.,* 2011).

India is predominantly an agricultural country and has the largest livestock population in the world. Livestock sector plays a critical role in livelihood security and the welfare of India's rural population. It contributes 9 % to GDP and employs 8% of the labour force. This sector is emerging as an important growth leverage of the Indian economy. It is sub-sector of agriculture which adds almost 32% of agriculture output in India. India supports 20% of the livestock population of the world on 2.3% geographical area only. The country face a net deficit of 61.1% green fodder, 21.9% dry crop residues and 64% feeds (Vision 2030, IGFRI). There is a large gap between requirement and availability of feed at the national level. It is matter of prime concern to bridge this gap. Most of the deficient regions lie in the arid and semi-arid agro-ecological zones.

Animal feed is the most crucial input in livestock production. Empirical studies in India have shown that enhancing quality and quantity of feed input has greater impact than breed improvement on increasing milk productivity (Gaddi & Kunal, 1996). Although India is the highest milk producer country but per capita milk production is very low due to the huge deficit in the availability of feed stuffs. This is also to note that area under forage production has not increased considerably in the last few decades and our natural grazing lands and pastures are fast degrading and decreasing. Hence, efforts should be directed to intensify forage production per unit area per unit time, which can be achieved through improved high yielding varieties/hybrids and better management practices. Sorghum has a significant role in livestock production, particularly in tropical zone where feed stuffs could not meet animal requirements due to many factors such as poor soil fertility and drought (Pholsen & Suksri, 2007).

Along with this forage quality is paramount to palatability or acceptability and animal intake. Plant morphology, anatomical components, digestibility, protein, mineral, cellulose and lignin contents, and anti-nutritional factors like hydrocyanic acid in sorghum determine animal performance like milk and meat production, (Hanna 1993). Lignin concentrations in brown-midrib (bmr) mutants are consistently lower than their normal counterparts in sorghum (by 21.8%). The *in vitro digestibility* of bmr sorghum (642 g kg^{-1} dry matter) was higher than the normal genotypes of sorghum (568 g kg^{-1} dry matter) (Cherney *et al.,*1988).

To realizing forage potential of sorghum there is an urgent need to develop multi-cut, intra-specific, single-cross, forage hybrids. These hybrids provide a better alternative to forage varieties grown during the summer and kharif season. So, present study was planned with the object to screen no. of hybrids for various morphological and biochemical parameters that determines forage yield and quality.

Materials and Methods

Hybrid evaluation experiments were conducted during *kharif* 2013 and 2014 at Research area of forage farm at CCS HAU, Hisar. Research material consisted of fifteen forage sorghum hybrids and check SSG 59-3 (a multicut variety) was sown in randomized block design to estimate overall performance of hybrids for two successive years. Fifteen MS based hybrids have been developed for two successive years and then evaluated for two years for various agronomic traits that affect the biomass production directly or indirectly.

Hisar is located at 29.09°N 75.43°E in western Haryana. Hisar has very hot summers and relatively cool winters. The maximum day temperature during the summer varies between 40 to 46°C. Relative humidity varies from 5 to 100 per cent. The average annual rainfall is around 350 mm most of which occurs during the months of July and August. Meteorological data of both the years during growing period was given in Table 1. The hybrid evaluation trial was sown in randomized block design having plot size 20$m^2$with row to row and plant to plant spacing 45cm and 30 cm, respectively for evaluating them along with check for various yields and forage quality related traits. Data were recorded on five randomly chosen plants for early vigor, TSS%, regeneration potential, plant height, number of tillers per plant, green (GFY) and dry fodder yield (DFY) after 1st and 2nd cut. First cut was taken after 60 days and then 2nd cut was taken after 45 days. For DFY, 500 g of green fodder was dried and then weighed to calculate DFY q/ha. For quality estimation samples of green fodder harvested from the field then heads were cut from the stalks and 500 g sample was weighed, and was dried to constant weight at 60 ºC for dry matter determination. Then dried sample was passed through a small chopper, mixed thoroughly, and sampled for dry matter determination and laboratory analyses. HCN µg/100g was estimated on fresh wt. basis by Gilchrist *et al*., 1967 method, crude protein (% N X 6.25) was estimated via modified Kjeldahl *et al*., 1883 procedure and IVDMD% was determined by the two-stage technique of Tilley and Terry, 1963 and expressed as a percentage. Data collected over the 2 years were subjected to analysis of variance, GCV, PCV, heritability and simple correlations using the appropriate software.

Results and Discussion

The means and ranges for all fodder yield and quality traits, plus the level of significance for the 16 sorghum genotypes studied, are reported in Table 2. Highly significant ($P<0.001$) differences among genotypes were ob-served for early vigor, TSS%, regeneration potential, plant height, number of tillers per plant, green (GFY) and dry fodder yield (DFY) after 1st and 2nd cut for both seasons. This indicates the prevalence of enough genetic variability in the material under study for selection and improvement of genotypes for biomass production. It also shows its suitability for further statistical analysis for all the characters under study. Among all hybrids HH 530, HH 561 had shown more plant height as compared

to check SSG 59-3. All the hybrids under study had shown good early vigor except HH 520 having early vigor score only one. HH 602 and HH 551 had shown good regeneration potential. Maximum no. of tillers per plant was observed in HH 676 and HH 561. In all the hybrids HCN content was below 200 µg/100g on fresh wt. basis (toxic limit is 200 µg/100g). Crude protein range from 6.9 (HH 520) to 9.8% (HH 619) and IVDMD % was from 39.9 (HH 539) to 55.6 (HH 619).

Similar results of outperformance of hybrids as compared to local checks and the commercial hybrids for forage yield were reported by (Mohammad *et al.,* 2012) although some morphological traits like number of tillers/plant, leaf length, leaf breadth, stem girth and number of leaves/plant all hybrids have almost equivalent to the checks. Pothisoong & Jaisil 2011 had evaluated twenty sweet sorghum F_1 hybrids for yield potential, heterosis and ethanol production and observed significant improvement for all traits in hybrids as compared to their parents. Akabri *et al.,* 2012; Goyal *et al.,* 2013 developed two hybrids Surat-1 x C-10-2, Surat-4 x UP Chari and 94002A x RSSV-9 and NSS1007A x Ramkel respectively showing high green fodder yield over commercial cultivars and checks. Pahuja *et al.,* 2014 observed outperformance of hybrids as compared to local checks in forage yield for all traits like number of tillers/plant, leaf length, leaf breadth, stem girth and number of leaves/plant. They had reported that hybrid 56A X COFS29 was unique in achieving high forage yield and choice of parents involved in hybrid production, especially female parent used should have good combining ability for forage yield.

In order to get enhanced performance of animals, the quality of fodder being fed to them is of utmost importance. The main quality attributes in forage sorghum are protein, IVDMD, NDF, ADF and toxic substances like HCN and tannin. Out of these proteins, IVDMD and toxic substances are most important. Like other straws, the nutritive value of sorghum fodder is also low due to presence of high content of above mentioned cell wall constituents as well as lignin and low content of protein and minerals. Crude protein (CP) content is often considered a good determinant of quality. Good quality forage generally will have higher protein content. Average 20% of crude protein is unavailable to ruminants due to tannin. Goal in breeding programme is to improve CP more than 9 %. IVDMD (*in vitro dry matter digestibility*) is a measure of plant quality index. Findings of present investigation is in close conformation with the findings of Grewal *et al.,* 1996 who revealed that protein and IVDMD varied from 3.01 to 8.75 and 40.40 to 66.16 %, respectively. Kumar *et al.,* 2011 reported that protein content in single cut (SC) and multicut (MC) genotypes ranged from 5.24 to 10.06 and 4.81 to12.47 per cent, respectively. R a n g e , genetic coefficient of variation (GCV), phenotypic coefficient of variation (PCV), heritability in broad sense (h^2bs) and genetic advance as percent of mean have been presented in Table 2.

Information on the nature and magnitude of genetic variability is of immense importance for initiating any breeding programme, because presence of considerable variability in the base material ensures better chances of evolving desired plant types. The estimates of PCV, GCV, heritability and genetic advance as per cent of mean are useful in determining the method of selection to improve a particular population for a specific trait. It is clear from Tables 1 that it was always not necessary for high heritability to be associated with high genetic advance. The genetic constants for the characters revealed that the magnitude of phenotypic coefficient of variation (PCV) was higher than the corresponding genotypic coefficient of variation (GCV) for all the traits denoting environmental factors influencing their expression to some degree or other. Wide difference between PCV and GCV implied their susceptibility to environmental fluctuation, whereas narrow difference suggested their relative resistance to environmental alteration. High magnitude of GCV and PCV suggested greater scope for selection of superior genotypes for these traits. In the material under study, wide range of variation, high coefficient of variation, heritability and genetic advance as per cent of mean were recorded for early vigor, no. of tillers per plant, regeneration potential, dry fodder yield, green fodder yield, and HCN. It may be due to the presence of additive gene action for these characters and hence, simple selection would be the most appropriate breeding method for their improvement. Similar results were also reported by Jain *et al.,* (2009) for number of leaves per plant, green fodder yield and dry fodder yield. Our results are in agreement with findings of Kumar & Sahib (2003) and Bello *et al.,* (2007). Tariq *et al.,* (2012) studied 25 sorghum genotypes and found high GCV and PCV for green fodder yield whereas our result are contrary to this in respect of no. of tillers, green fodder yield, dry fodder yield and HCN had high heritability estimates along with high genetic advance. Traits like plant height, crude protein and IVDMD%, revealed high heritability associated low

genetic advance as percent of mean. This may be due to presence of both additive as well as non additive type of gene action. Similarly Saini & Paroda (1975) reported high heritability for protein and medium heritability for IVDMD.

Most of the agronomic traits evaluated in this study showed positive and significant ($P<0.05$) correlation among themselves (Table 3). For instance, there was also a positive and significant correlation between number of tillers per plant and plant height (0.575), plant height and green fodder yield (0.657), green fodder yield and regeneration potential (0.549), green fodder yield and dry fodder yield (0.903), dry fodder yield and plant height (0.609) and IVDMD and CP was 0.707. The significant positive correlation among these traits suggests that these traits could be simultaneously improved without any compensatory negative effects. However, negative and significant correlation was observed among regeneration potential and IVDMD% (-0.554). The negative relationship between these traits suggests that they should be improved independently. Because forage sorghum breeding program should aim for improvement of important fodder quality traits, such as digestibility and protein content, in addition to forage yield. Similar results for the association of significant correlation between number of days to flowering, number of grains per panicle, number primary panicles, number of days to maturity, panicle weight and biological yield was reported by Nyadanu & Dikera (2014). Mahajan *et al*., 2011 and Millinath *et al*. 2004 also reported that days to 50% flowering, panicle length, plant height and number of grains/panicle were associated among themselves in sorghum.

Considering morphological characters, both green and dry fodder yield are equally important, however, we have evaluated hybrids thus we consider green fodder yield as important trait. Thus, in present investigation, path coefficient analysis was performed considering green fodder yield as dependent character. Residual effect calculated was 0.0938 (Table 4). This indicates that a considerable magnitude of variation was presented for association of green fodder yield with dependent traits. Path coefficient analysis was carried out among six variables including morphological and quality traits which revealed that plant height, dry fodder yield and CP% exhibited high positive and direct effect on green fodder yield. While number of tillers per plant had positive but indirect effect on green fodder yield *via* plant height.

Our results are similar to Iyanar and Khan (2005) who has found plant height had highest direct effect on green fodder yield followed by leaf breadth. Jadhav *et al*., 2009 found only green fodder yield/plant had high direct contribution on dry fodder yield. Whereas, leaf length, leaf width, plant height and stem thickness had high positive indirect contribution on dry fodder yield through green fodder yield. Hence, the present investigation emphasized that male sterility based hybrids may help in formulation of breeding strategies for improvement of forage sorghum for the green as well as dry fodder yield along with quality traits viz., protein, digestibility and for low HCN. Thus efforts should, therefore, be directed at designing forage sorghum varieties/hybrids that tiller, grow tall and ensure multi-cuts. For that there is an urgent need to develop a multidisciplinary approach and multi-institutional alliances of crop scientists, chemical technologists, and animal health and nutrition experts having a major role in good quality forage research and cultivar development.

Table 1. Meteorological data during the growing periods.

Year and Month	Temperature (ºC)		Relative humidity (%)		Rainfall (mm)
2013	**Max**	**Min**	**Morning**	**Evening**	**Total**
July	36.1	26.9	83.4	61.8	147.7
August	33.4	25.7	89.2	70.2	299.7
September	34.3	23.8	84.8	54.7	144.4
2014					
July	37.64	27.7	71.4	50.6	49.9
August	36.5	26.2	78.7	50.2	1
September	34.9	23.7	83.0	50.7	81.5

Table 2. Overall mean, range, GCV, PCV, heritability and genetic advance as per cent of mean for different characters among MS based hybrids

S. No.	Variables	Mean	Range	Coefficient of variations (%)		Heritability (%)	Genetic advance as % of mean
				GCV	PCV		
1	Early Vigor	3.0	1.0-4.0	31.98	31.98	100	65.5
2	TSS	3.8	3.0-5.0	17.49	18.53	89	33.5
3	Regeneration	2.9	1.0-5.0	47.3	47.3	100	96.5
4	Plant Height (cm)	223.6	166.3-267.0	15.48	15.93	93.5	30.5
5	No. of tillers	3.5	2.0-5.8	36.64	40.67	78	67
6	Green fodder yield (q/ha)	649.8	400.0-879.5	20.49	20.56	98.5	41.5
7	Dry fodder yield (q/ha)	147.8	89.8-243.8	28.55	29.18	94.5	57
8	HCN	8.1	11.8-124.7	60.97	62.61	94	121.5
9	Crude protein%	47.8	6.9-9.8	11.43	12.53	82.5	21
10	IVDMD%	50.5	39.9-55.6	9.25	11.98	59	14

Table 3. Correlation coefficient analysis observed among various morphological traits among MS based hybrids

Variables	Early vigour	TSS%	Regeneration	Plant Height (cm)	No. of tillers	Green fodder yield (q/ha)	Dry fodder yield (q/ha)	Crude protein%	IVDMD %
IVDMD%									1
Crude protein%								1	**0.707****
Dry fodder yield (q/ha)							1	0.437^{NS}	0.186^{NS}
Green fodder yield (q/ha)						1	**0.903****	0.450^{NS}	0.110^{NS}
No. of tillers					1	0.411^{NS}	0.320^{NS}	0.233^{NS}	0.021^{NS}
Plant Height (cm)				1	**0.575***	**0.657****	**0.609***	0.418^{NS}	0.365^{NS}
Regeneration			1	0.039^{NS}	0.149^{NS}	**0.549***	0.302^{NS}	-0.113^{NS}	**-0.554***
TSS %		1	-0.039^{NS}	**0.758****	0.163^{NS}	0.402^{NS}	0.315^{NS}	0.370^{NS}	0.376^{NS}
Early vigour	1	0.146^{NS}	0.243^{NS}	0.217^{NS}	-0.003^{NS}	0.401^{NS}	0.366^{NS}	0.362^{NS}	0.151^{NS}

Table 4. Showing direct (In diagonal) and indirect effect among MS based hybrids for various morphological traits (Residual effect: 0.0938)

Variables	Plant Height (cm)	No. of tillers	Green fodder yield (q/ha)	Crude protein%	IVDMD%	Dry fodder yield (q/ha)
Plant Height (cm)	**0.464**	0.27687	0.00338	0.00338	0.20277	0.28556
No. of tillers	0.46366	-0.063	-0.01976	-0.01976	-0.01342	-0.021
Green fodder yield (q/ha)	-0.03753	-0.06285	**1**	0.24846	0.07369	0.0648
Crude protein%	0.00181	0.07813	0.24846	**0.248**	-0.32855	-0.0324
IVDMD%	-0.14369	-0.07016	-0.09744	-0.09744	**-0.329**	0.6094
Dry fodder yield (q/ha)	0.37532	0.20367	0.15893	0.15893	0.06009	**0.609**

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Research Article

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Genome-Wide Association Mapping Using a Bayesian Mixture Model for Plant Height in *Oryza sativa*

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ABSTRACT

Genotypic and phenotypic data could be used to predict inheritance of complex traits for plant breeding in genome wide association mapping studies (GWAS). In GWAS using a single marker model may leads to suboptimal use of genotypic datasets. Alternatively, using whole genome, a Bayesian mixture model may cluster markers into predefined classes. We used 413 diverse accessions of *Oryza sativa* with 36900 Single Nucleotide Polymorphisms (SNPs) markers for plant height. We assumed different genetic architectures for the phenotype. We estimated genotypic heritability as 0.61. Bayesian mixture model detected 144, 446, 54 SNPs with explanatory levels of 0.0001, 0.001 and 0.01 respectively. Chromosome 1 (n=109), and 3 (n=85) had the highest explanatory genetic variances as 23% and 19%, respectively. Correlation between genomic predicted observations and actual observations was found to be 0.94. Since GWAS are mostly based on only one replication as was also the case in this study; results need to be confirmed by independent validation experiments.

Keywords: Genome wide association mapping studies, Bayesian mixture model, Single Nucleotide Polymorphisms Markers, *Oryza sativa*.

Introduction

Plant height is an important complex yield related trait in *Oryza sativa*. However, height itself is an important model phenotype in various organisms. Plant height is easy to measure, highly heritable but underlying biology is found to be complex.

Genotypic and phenotypic data could be used to predict complex traits for plant breeding in genome wide association mapping studies (GWAS) . Single Nucleotide Polymorphisms (SNPs) could be used as markers to detect genomic signals over chromosomes in GWAS. Although GWAS found genomic signals for various phenotypes in various organisms, explanatory proportion of the SNPs remained very low. This unexpected result termed as missing heritability problem (Turkheimer, 2011). For example only 5% of the height phenotypic variance explained by about 50 variants using human GWAS (Yang *et al*., 2010).

Moser *et al*., (2015) suggested to use a bayesian mixture model to overcome associated problems in GWAS including multiple hypothesis testing, linkage disequilibrium and for increasing the power of the experiment. Employing whole SNPs altogether (Meuwissen *et al*., 2001) could be beneficial compared with marker assisted selection (MAS) that employs a few of the markers at the selection stage. Main aim of this study was genome wide association mapping of plant height in *Oryza sativa* using both single SNPs and a bayesian mixture models (Moser *et al*., 2015).

Materials and Methods

Data

The GWAS included 413 Asian rice cultivars from 82 countries. The genome consisted 36900 SNPs distributed over 12 chromosomes. Plant height was measured as distance between from tip of main panicle of the plant to soil surface. More details about the dataset could be found at Zhao *et al*., (2011).

Genome wide Rapid Association Using Mixed Model and Regression

One of the important GWAS assumption is that the

individuals in the samples should be unrelated. Genetic and geographic relationship among the individuals may lead to deviation from this assumption. Aulchenko *et al*., (2007) defined a mixed model to correct for genetic (or when the pedigree file is not available, genomic) effects: Genome wide Rapid Association Using Mixed Model and Regression (GRAMMAR). We used a genomic relationship matrix in the GRAMMAR to correct for relation between plant samples. In addition we obtained first four principal components from the samples to correct for geographical relationship among the samples. In GRAMMAR $y=Xb+Zu+e$ used, where X and Z are design matrices to link plant height (y: response variable) with fixed *b* (top four principal components) and random *u* effects (additive gene effects:), *e* and *u* (weighted with genomic or pedigree information) was assumed to be sampled from normal distribution and their respective variance components was predicted using maximum likelihood procedure as was implemented in GenABEL (Aulchenko *et al*., 2007). We used a single SNP regression model using the GRAMMAR approach by GenABEL to detect associated genes with plant height.

A Bayesian mixture model

We used a hierarchical bayesian mixture model (Moser *et al*., 2015) (BayesR) to obtain SNP solutions by using whole genome simultaneously. BayesR assumed a mixture of four normal distributions for the SNP effects to be predicted (assumed to be 0.00001, 0.0001, 0.001, 0.01). We sampled 50000 markov chains and discarded first 20000 as burn in period and recorded every 10th sample for thinning the chain. We compared results of different runs of markov chains to assess convergence.

Results and Discussion

We excluded 8214 SNPs based on minor allele frequency of <5%, and call rate < 90% leaving 28686 SNPs in the dataset. We excluded 259 individuals due to too high identity by state (IBS) (0.95>) leaving 154 individuals in the analyses. Mean IBS estimated as 0.62 (0.15) and mean autosomal heterozygosity estimated as 5.91e-05 (4.49e-05). Genomic heritability was found to be 0.61.

GRAMMAR approach identified strong signals from various locations of the genome (Table 1) however after multiple hypothesis testing only from chromosome 6 (id6002498) had a suggestive genomic signal that could be detectable (Figure 1).

Quantile-quantile plot (Figure 2) showed that still there might be indication of population stratification as inflation factor was found to be 1.34 (0.0001).

We used both full data set (n=413, data1) and quality control filtered data set (n=154, data2) to investigate impact of population sub structure (mainly due to geographical and genetic relationships of the individuals) to the Bayesian mixture analyses. Since we used different proportion of explanatory variances for each sub classes we could investigate SNP effects from chromosomes and/ or from certain loci. We presented the results of BayesR in Table 2 using both Data1 and Data2.

When we filtered out the highly correlated individuals (Data2) BayesR detected 3376 SNPs compared with the full data set (Data1) as 644 SNPs. .

Table 2 suggested that the highest explanatory proportions obtained from chromosome 1 as 0.23 and 0.19 for Data1 and Data2 respectively. Bayesian mixture model detected 144, 446, 54 SNPs with explanatory levels of 0.0001, 0.001 and 0.01 using Data1. Bayesian mixture model detected 2957, 356, 155 SNPs with explanatory levels of 0.0001, 0.001 and 0.01 using Data2.

We detected mostly small SNPs effects from various part of the genome using both Data1 (Table 3) and Data 2 (Table 4). Correlation between genomic predicted observations and actual observations found to be 0.94 and 0.99 for Data1 and Data2 respectively. As was expected using homogenized samples (Data2) lead to higher accuracy for predicting phenotypes using genotypic information. Since the GWAS are mostly based on only one replication (as was also the case in this study); results needs to be confirmed by independent validation experiments.

Conclusion

Employing homogenized sample in GWAS using IBS information to overcome population stratification leads to better genomic predictions of plant height for *Oryza sativa* by the Bayesian mixture model.

Acknowledgment

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Table 1. Summary of genome wide rapid association using mixed model and regression (P: raw p values, Pc corrected p values using 1000 permutations)

SNP	Chromosome	Chi square	P	Pc
id6002498	6	37.76	7.97E-10	0.077
id11006324	11	27.22	1.81E-07	0.549
id12000343	12	25.98	3.44E-07	0.66
id2001384	2	25.36	4.74E-07	0.706
id1020583	1	24.94	5.91E-07	0.739
id1020512	1	24.53	7.31E-07	0.773
id1020569	1	24.53	7.31E-07	0.773
id1020642	1	24.53	7.31E-07	0.773
id3013805	3	23.73	1.11E-06	0.827
id6010525	6	23.62	1.17E-06	0.831

Figure 1. Manhattan plot of GWAS result using GRAMMAR approach. The x-axis of the Manhattan plot shows the genomic position, the y-axis represents the log10 base transformed p-values.

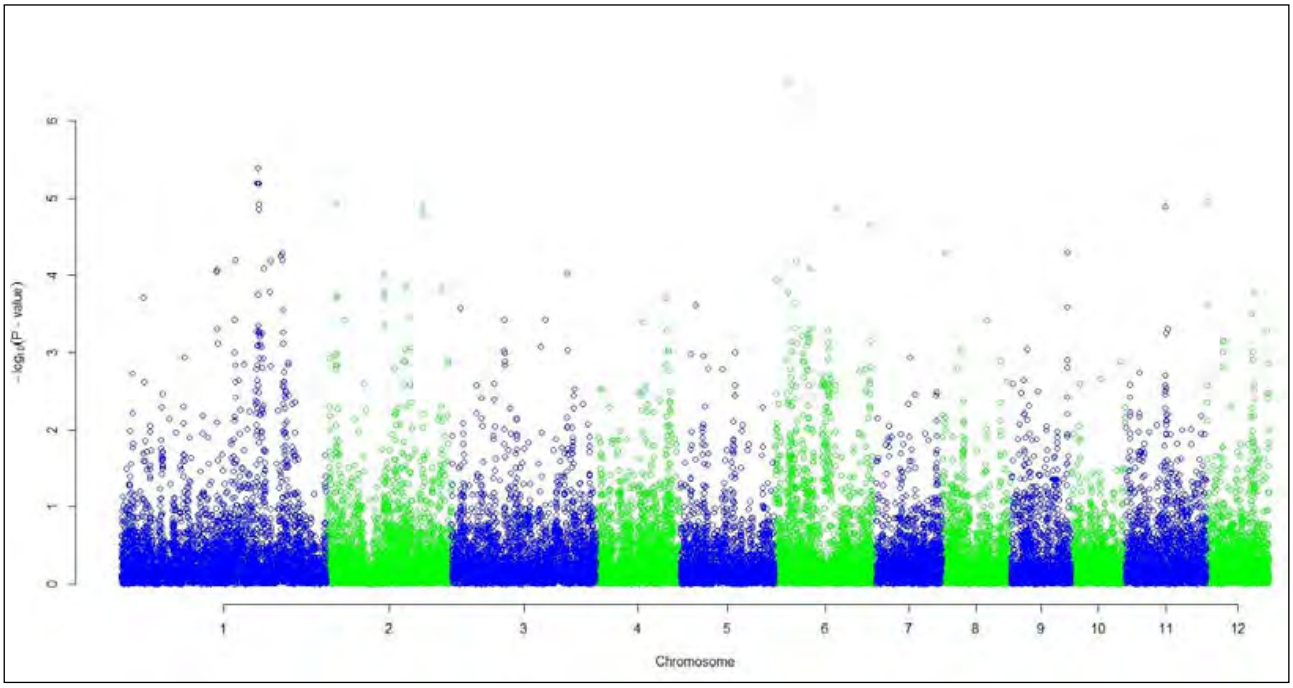

Figure 2. Quantile-Quantile plot of GRAMMAR GWAS result.

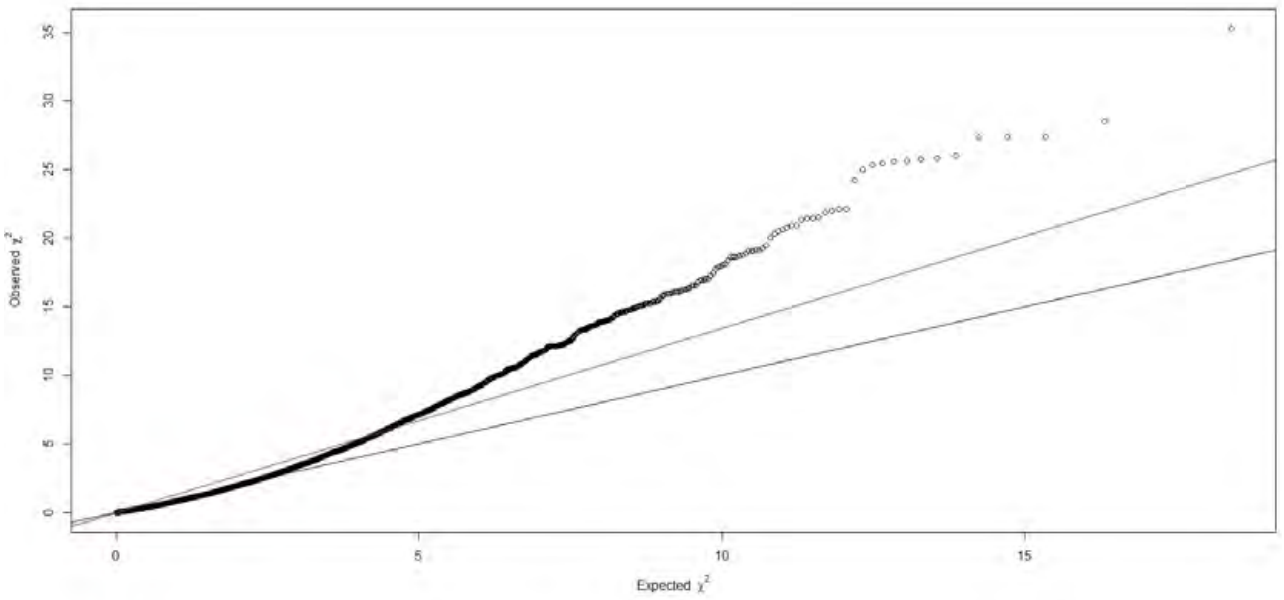

Table 2. Summary of Bayesian mixture model analyses for full data (Data1) and reduced data (Data2).

	Data1 (n=413)		Data2 (n=154)	
Chromosome	**%Genetic Variance**	**# of SNPs**	**%Genetic Variance**	**# of SNPs**
1	0.23	108	0.19	583
2	0.07	69	0.14	377
3	0.19	85	0.15	418
4	0.03	52	0.06	241
5	0.09	45	0.11	296
6	0.14	63	0.12	308
7	0.03	43	0.08	174
8	0.02	36	0.07	187
9	0.05	38	0.09	203
10	0.05	29	0.03	162
11	0.07	39	0.09	240
12	0.05	37	0.06	187

Table 3. Summary of Bayesian mixture genome wide association model for Data1.

Chromosome	SNP	Base Pair	Effect	%Genetic Variance
1	dd1000754	7334172	12.30	0.06
3	id3016879	34446028	9.34	0.05
5	id5013556	27621664	9.07	0.04
3	id3001242	2224752	6.63	0.03
10	id10001390	4682100	6.03	0.03
6	id6002006	2655955	5.65	0.03
1	id1024441	38537795	5.61	0.03
1	id1018291	30408458	5.26	0.03
3	id3016453	33688227	4.92	0.02
6	id6006541	10552135	4.89	0.02

Table 4. Summary of Bayesian mixture genome wide association model for Data2.

Chromosome	SNP	Base Pair	Effect	%Genetic Variance
11	id11007149	18919417	11.48	0.03
7	id7004642	25018437	5.49	0.02
1	id1018227	30312328	5.37	0.02
2	id2003308	6479823	4.86	0.01
2	id2005948	14158673	4.70	0.01
9	id9005931	17645328	3.98	0.01
6	id6009548	16836564	3.92	0.01
5	wd5001329	10805291	3.80	0.01
1	id1028014	42619920	3.57	0.01
2	id2011435	26085576	3.36	0.01

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Genetic Analysis of some Important Quantitative Traits in Bread Wheat (*Triticum aestivum* L.)

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ABSTRACT

The estimation of gene effects in the inheritance of yield components is one of the most important tasks in wheat breeding programs. The objective of this study was to estimate gene effects for the number of grains and grain weight per spike in ten wheat crosses with five basic generations: parent cultivars (P1, P2), first and second filial generations (F1, F2) and first backcrosses (BC1). The gene effects responsible for inheritance of these two important traits were estimated on the basis of generation mean analysis, using an additive-dominant model with three and six-parameters. The adequacy of the additive-dominance model with three-parameters was tested using the Scaling test and Chi-square ($\chi 2$) test. A three-parameter model was found as adequate to explain variation for the both traits in three crossing combinations. The magnitude of additive gene effects (d) was smaller than the corresponding dominance effects (h) in most crosses for both traits. The application of six-parameter models indicated the significant epistatic effects for explaining genetic variation for these traits. The inheritance of these traits was influenced by additive×additive (i) and dominance×dominance (l) type of non-allelic interactions in the study. Duplicate gene interactions were also observed in most crosses of both characters which are difficult to exploit in breeding programs. These results implied that the selection for the improvement of these traits should be applied in further generations in wheat breeding..

Keywords: Wheat, Additive-dominance model, Gene effects.

Introduction

Wheat is one of the major cereal crops in the World, grown on over 220 million hectares, representing 26% of the total harvested area, on average (USDA, 2015). Considering growing demands rising approximately 2% per year, which is twice of the current gain rate in genetic yields potential, plant breeders have to put many efforts to improve the grain yield of wheat (Reynolds *et al*., 2001). Wheat grain yield is a complex polygenic trait influenced by many components and can be improved through indirect selection on the basis of yield components. The increment in one component might have positive or negative effect on the other components (Chandra *et al*., 2004). This is the reason why it is necessary to know the genetic architecture of yield components. Therefore, information about the nature, magnitude of gene effects and their contribution to the inheritance of the yield and yield components is essential to plant breeders for improving wheat grain yield (Petrović *et al*., 2001). In order to get information about gene action that is controlling the traits, plant breeders often use generation mean analysis. Generation mean analysis is a useful technique in plant breeding for estimating main gene effects (additive and dominance) and their digenic (additive×additive, additive×dominance, and dominance×dominance) interactions responsible for inheritance of quantitative traits (Singh and Singh, 1992; Kearsey and Pooni, 1996). As the number of grains and grain weight per spike are important quantitative traits of wheat which directly affects the yield, a large number of genetic studies have been made to investigate the genetic basis

of these traits of wheat. The importance of epistatic effects in controlling these traits was observed by many researches (Dvojković *et al*., 2010; Zaazaa *et al*., 2012; Ijaz and Kashif., 2013).

The present study was carried out to obtain information about the types of gene effects for the number of grains and grain weight per spike of different bread wheat genotypes.

Materials and Methods

Five, diallely crossed, winter wheat varieties (*Triticum aestivum* L.), namely, Pobeda, Renesansa, Sara, Partizanka and Pesma, were used in the study. Five plant material categories were obtained: parental varieties (P_1, P_2), progenies (F_1, F_2) and back-crosses (BC_1) of ten cross combinations (Pobeda/Renesansa, Pobeda/Sara, Pobeda/Partizanka, Pobeda/Pesma, Renesansa/Sara, Renesansa/Partizanka, Renesansa/Pesma, Sara/Partizanka, Sara/Pesma and Partizanka/Pesma). The trial was sown in a randomized block design, with three replications, during the three successive seasons of 2009/2010, 2010/2011 and 2011/2012. Field experiment was conducted at the experimental field of the Institute of Field and Vegetable Crops in Novi Sad, Serbia. The cultivars were sown in 2 m long rows with 20 cm of inter-row spacing and 10 cm spacing between plants in the row. The main sample consisted of 10 plants per replication. At the stage of full maturity grain yield components, namely the number of grains per spike and grain weight per spike (g) were analyzed. The gene effects of the grain yield components, number of grains and grain weight per spike of wheat, were estimated for each cross combination by Generation mean analysis (P_1, P_2, F_1, F_2 and BC_1), using an additive-dominance model of three-parameters (Mather, 1949). The adequacy of the additive-dominance model was tested using the Scaling-test (A, B and C) and Chi-square ($\chi 2$) test. The simple genetic model (m, d and h) was applied when epistasis was absent, whereas in the presence of non-allelic interaction the analysis was proceeded to estimate the interaction types involved using the six-parameter genetic model i.e. (m, d, h, i, j and l) according to Mather and Jinks (1982). According to the methodology of Hayman (1960) the following notation for gene effects were used, where (m) represents mean effect, (d) additive gene effects, (h) dominance gene effects, (i) additive×additive epistatic effects, (j) additive×dominance epistatic effects and (l) dominance×dominance epistatic gene effects. The type of epistasis was determined only when dominance (h) and dominance×dominance (l) effects were significant. When these effects had the same sign, the type of epistasis was complementary, while different signs indicated duplicate epistasis (Kearsey and Pooni, 1996).

Results and Discussion

The additive-dominance model with three-parameter revealed that dominance effects (h) showed higher values than the additive effects (d) for number of grains per spike, indicating that dominance gene effects play the major role in controlling the genetic variation of this trait for all crosses. These results are in accordance with results reported by Petrović (1995) and Fethi and Mohamed (2010). The results of applying Scaling test and Chi-square (χ^2) test showed that the additive-dominance model, with three-parameter, was sufficient to explain genetic variation for the number of grains per spike for three crosses: Renesansa/Sara, Renesansa/Partizanka and Partizanka/Pesma. The results of Scaling test indicated that each of A, B or C was significant or highly significant for the number of grains per spike in the remaining seven crosses: Pobeda/Renesansa, Pobeda/Sara, Pobeda/Partizanka, Pobeda/Pesma, Renesansa/Pesma, Sara/Partizanka and Sara/Pesma. Thus, it indicated the presence of non-allelic gene interaction for these crosses and revealed that simple model with three-parameter is inadequate for explaining the inheritance of number of grains per spike (Table 1.).

In controlling inheritance for the grain weight per spike, the additive-dominance model with three-parameter revealed that both additive and dominance gene effects were important, with prevalence of dominance gene action, which is in accordance to the results reported by Dvojković *et al*., (2010). For the grain weight per spike, Scaling test and Chi-square (χ^2) test showed that the three-parameter model, was sufficient to explain genetic variation for three crosses: Pobeda/Sara, Renesansa/Sara and Sara/Partizanka, while in the remaining cross combinations three-parameter model failed and was found to be inadequate to explain genetic variation the inheritance of this trait (Table 1).

Therefore, the six-parameter model was applied and was fitted for explaining genetic variation for both traits. The estimates of the six parameters, i.e. means (m), additive (d), dominance (h), additive×additive (i), additive×dominance (j) and dominance×dominance (l) are presented in Table 2.

Using the six-parameter model it was observed that the mean effects were highly significant for both traits in the most crosses, indicating that these traits are quantitatively inherited. For the number of grains per spike it confirmed the presence of significant epistatic effects in all cross combinations, except the three cross combinations: Pobeda/Renesansa, Renesansa/Partizanka and Partizanka/Pesma. In the cross combination Pobeda/Renesansa, Scaling test in previous model, indicated the presence of epistasis, while those are not determined, which suggested the presence of three-genic or polygenic epistasis. Similiar results were reported by Mather and Jinks

(1982) and Sharma *et al*., (2012). Also, it was revealed that the magnitude of additive gene (d) effects were slightly smaller relative to the corresponding dominance effects (h) in most cases, indicating that dominance gene effects play the major role in controlling the genetic variation of the number of grains for all crosses. These results suggesting that in these crosses pedigree selection method is a useful breeding program for improving these populations. The importance of dominance effects in controlling of the number of grains was observed by Fethi and Mohamed (2010). On the contrary, according to Dvojković *et al*., (2010), additive effects predominate in controlling the number of grains. The prevailing type of non-allelic gene interactions which was observed in many crosses was additive × additive (i) and dominance×dominance (l). Additive × additive type of non-allelic gene interactions were noticed in cross combinations: Pobeda/Sara, Pobeda/Partizanka, Renesansa/Pesma and Sara/Pesma. Dominance × dominance type of non-allelic gene interactions were noticed in cross combinations: Pobeda/Pesma, Renesansa/Sara, Renesansa/Pesma, Sara/Partizanka and Sara/Pesma. In crosses Pobeda/Pesma, Renesansa/Sara, Renesansa/Pesma and Sara/Pesma duplicate type of non-allelic interaction was apparent since dominance effects (h) and dominance×dominance epistatic effect (l) were significant and in opposite sign. In this case success of the selection would be affected negatively by these interactions. The presence of duplicate epistasis is unfavorable from the breeder's point of view because it causes decreasing effect on the analyzed trait. Duplicate epistasis in the number of grains inheritance has been reported also by Erkul *et al*., (2010) and Ijaz and Kashif (2013). Contrary to these results, the presence of non-allelic gene interaction caused by complementary genes was indicated (Novoselović *et al*., (2004). In the cross combinations: Pobeda/Pesma, Renesansa/Pesma and Sara/Pesma the less favorable case of duplicate type of epistasis was observed as the sign of the value of epistatic effects dominance×dominance (l) were negative, which causes reducing the effects of dominant gene and decreasing phenotypic expression of the trait. These results are less favorable for breeders, than if the values of dominance × dominance epistatic effects (l) were positive, as the crosses Renesansa/Sara. In this cross combination epistatic effect in a small amount masked the phenotypic expression of the trait. The presence of significant epistatic effects additive × additive (i), which has been observed in cross combination: Pobeda/Sara, Pobeda/Pesma, Renesansa/Pesma and Sara/Pesma, is more favorable for breeders as these effects increase the ability for successful selection of more superior genotypes. However, when non-additive effects are larger than additive, the improvement of the trait needs intensive selection through later generation. In cross combinations: Pobeda/Sara, Pobeda/Pesma and Sara/Pesma fixable additive gene effect was not significant, yet significant epistatic effects additive × additive (i) was observed which could be a result of some preferred interaction between the genes which are controlling this trait. The favorable situation was observed in the cross combinations Pobeda/Sara and Pobeda/Partizanka, considering in this crosses only epistasis additive × additive (i) significantly controlled the inheritance of the number of grains and this effect additionally draws gene effects in the direction to the additivity. When additive effects are larger than the non-additive, it is suggested that selection in early segregating generations would be effective. The results of six-parameter model also indicated that epistasis was not found in the inheritance of the number of grains for the crosses Pobeda/Renesansa, Renesansa/Partizanka and Partizanka/Pesma, which suggested that for this crosses an additive-dominance model was adequate. Absence of epistatic effects in these crosses greatly makes easier the selection for this trait, considering that the presence of epistasis complicated procedures for improving quality of traits. The results obtained here, revealed the importance of epistatic effects in the inheritance of the number of grains per spike and should not be ignored in establishment a new breeding program to improve wheat genotypes for this trait.

Regarding to the grain weight per spike, the six-parameter model was fitted for explaining genetic variation for the grain weight per spike and it confirmed the presence of significant epistatic effects in cross combinations: Pobeda/Renesansa, Pobeda/Pesma, Renesansa/Pesma and Sara/Pesma. The inheritance of the grain weight per spike was differing depending on cross combination and it was controlled by additive and non-additive gene effects. This indicated that both gene effects were equally important in controlling the genetic variation of the grain weight. This result is in accordance to the results reported by Dvojković *et al*., (2010). On the contrary, according to Zaaza *et al*., (2012) additive genetic variation predominates in the inheritance of this trait. The type of non-allelic gene interactions, which had been observed in many crosses, were additive×additive (i) and dominance×dominance and were noticed in cross combinations: Pobeda/Renesansa, Pobeda/Pesma, Renesansa/Pesma and Sara/Pesma (Table 2.)

In crosses Pobeda/Renesansa, Pobeda/Pesma and Renesansa/Pesma duplicate type of non-allelic interaction was confirmed since dominance effects (h) and dominance × dominance epistatic effect (l) were significant and in opposite sign. In this case success of the selection would be affected negatively by these interactions and causes decreasing effect on the analyzed trait.

Also in these crosses the less favorable case of duplicate type of epistasis, was observed as the sign of the value of epistatic effects dominance×dominance (l) were negative, which causes decreasing phenotypic expression the trait and effect of dominant gene effects. Duplicate epistasis in the inheritance grain weight has been reported also by Zaazaa *et al*., (2012) and Dvojković *et al*., (2010). Contrary to these results, where non-allelic gene interactions have been found in the inheritance of grain weight per spike as revealed by Garole and Monpara (2005) and Munir *et al*., (2007). Complementary type of epistasis was only found in the cross combination Sara/Pesma, as the significant dominant gene and significant epistatic effect dominance×dominance had the same sign. This situation is more favorable than the presence of duplicate type of epistasis due to a greater chance of breeding success. The presence of non-allelic gene interaction caused by complementary genes for the grain weight was also reported by Novoselović *et al*., (2004). The presence of significant epistatic effects additive×additive (i) which has been observed in this cross combination is more favorable for breeders as these effects increases the ability to successfully selection superior genotypes. This type of epistasis significantly controlled the inheritance of the grains weight and additionally draws gene effects in the direction to the additivity. In cross combination Renesansa/Pesma the negative sign of additive × dominance (j) interaction was observed and in most cases suggested dispersion of genes in the parents. These results are in agreement with those obtained by Khattab *et al*., (2010). The results of six-parameter model also indicated that epistasis wasn't found in the inheritance of the grain weight at the crosses: Pobeda/Sara, Pobeda/Partizanka, Renesansa/Sara, Renesansa/Partizanka, Sara/Partizanka and Partizanka/Pesma. This suggests that for this crosses an additive-dominance model was adequate, which greatly makes easier the selection for this trait, considering that the presence of epistasis complicated procedures for improving quality of traits.

Conclusions

In light of the present findings it can be concluded that the examined traits in this study have shown complex genetic behavior. The inheritance of the number of grains per spike was controlled by additive and non-additive genetic effects, with prevalence of dominance gene action in the most crosses. The results also revealed the importance of epistatic effects (additive × additive and dominance × dominance) in the inheritance of the number of grains per spike in cross combinations: Pobeda/Sara, Pobeda/Partizanka, Pobeda/Pesma, Renesansa/Sara, Renesansa/Pesma, Sara/Partizanka and Sara/Pesma. Therefore, selection in the advanced generations might be effective for number of grains due to dominance and epistatic effects.

The inheritance of the grain weight per spike was differing depending on cross combination and revealed that both additive and non-additive gene effects were important in controlling the genetic variation of the grain weight per spike of wheat. The results also revealed the importance of epistatic effects (additive×additive and dominance × dominance) in the inheritance of the grain weight per spike in cross combinations: Pobeda/Renesansa, Pobeda/Pesma, Renesansa/Pesma and Sara/Pesma. Therefore, breeding strategies which can exploit additive as well as non-additive gene effects could be used for improving these traits of wheat yield.

Acknowledgment

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Table 1. The estimates of gene effects for the number of grains and grain weight per spike using the three-parameter model in ten winter wheat crosses

Gene effect	Pobeda/Renesansa		Pobeda/Sara		Pobeda/Partizanka		Pobeda/Pesma		Renesansa/Sara	
	Crossing combinations									
	Trait									
	NG	GW	NG	GW	NG	GW	NG	GW	NG	GW
m	42.12	1.88	39.35	1.79	35.41	1.63	38.67	1.65	38.95	1.80
d	0.50	0.03	-0.09	-0.13	-1.86	-0.13	-0.81	-0.15	0.08	-0.07
h	1.46	-0.13	1.71	0.15	6.24	0.02	3.90	0.09	1.37	-0.15
	Scaling test									
A	6.27	0.40*	-0.08	0.10	-8.25*	0.34*	-0.94	0.11	-6.71	-0.36
B	5.79	0.34*	0.03	0.14	-2.84	0.03	1.37	0.44*	-6.13	-0.26
C	13.13*	-0.15	-17.61**	-0.32	-25.59**	0.75**	-15.48*	-0.40	-4.56	-0.09
χ^2 (3)	8.49*	13.63**	13.6**	2.26	36.27**	13.74**	10.91*	3.14**	7.41	4.48
P probability	<0.05	<0.01	<0.01	>0.01	<0.01	<0.01	<0.01	<0.01	>0.05	>0.01
Gene effect	**Renesansa/ Partizanka**		**Renesansa/Pesma**		**Sara/Partizanka**		**Sara/Pesma**		**Partizanka/Pesma**	
	Crossing combination									
	Trait									
	NG	GW	NG	GW	NG	GW	NG	GW	NG	GW
m	37.62	1.69	41.57	1.75	35.58	1.57	41.48	1.85	36.17	1.46
d	-2.29	-0.17	-1.44	-0.16	-1.76	-0.06	-0.79	-0.23	1.34	-0.02
h	2.59	-0.06	3.47	0.08	4.94	0.31	5.12	0.08	3.11	0.18
	Scaling test									
A	-1.40	-0.37	14.50	0.38*	-13.41	-0.40	14.08**	14.08**	1.28	-0.08
B	4.50	0.01	17.28	0.78**	-8.10*	-0.13	16.28**	16.28**	-1.83	-0.06
C	-2.21	-1.01*	2.04	0.14	-17.90**	-0.60	0.14	0.14	-2.12	-0.57
χ^2 (3)	3.00	10.65*	42.08**	8.55**	25.21**	5.15	50.58**	50.58**	0.56	9.31*
P probability	>0.05	>0.01	<0.01	<0.01	<0.01	>0.01	<0.01	<0.01	>0.05	>0.01

NG=Number of grains, GW=Grain weight,
*Significant at 0.05, ** Significant at 0.01.

Table 2. The estimates of gene effects for the number of grains and grain weight per spike using the six parameter model in ten winter wheat crosses

	Crossing combinations									
Gene effect	**Pobeda/Renesansa**		**Pobeda/Sara**		**Pobeda/Partizanka**		**Pobeda/Pesma**		**Renesansa/Sara**	
	Trait									
	NG	GW	NG	GW	NG	GW	NG	GW	NG	GW
m	41.55**	0.97**	22.63**	1.20**	23.04**	1.28**	23.21**	0.70*	48.71**	2.34**
d	-0.24	-0.03	0.05	0.02	2.71	0.16*	1.15	0.16*	0.29	0.05
h	10.46	2.40**	37.42*	1.61	24.29	0.48	36.65**	2.54**	-28.41*	-1.77
i	-1.06	0.89	17.57*	0.61	14.50*	0.39	15.89**	0.95**	-8.27	-0.50
j	0.48	0.06	-0.11	-0.04	-5.41	-0.31	-2.30	-0.33	-0.58	-0.11
l	-11.01	-1.64**	-17.52	-0.85	-3.41	-0.02	-16.34*	-1.50**	21.11*	1.12
	Crossing combination									
Gene effect	**Renesansa/ Partizanka**		**Renesansa/Pesma**		**Sara/Partizanka**		**Sara/Pesma**		**Partizanka/Pesma**	
	Trait									
	NG	GW	NG	GW	NG	GW	NG	GW	NG	GW
m	32.47**	1.02**	9.60	0.67**	41.11**	1.58**	8.82	0.01	34.81**	1.07**
d	2.95**	0.19**	1.39*	0.20**	2.65*	0.14	1.10	0.15	-1.56	0.01
h	15.94	1.14	92.50**	3.16**	-22.08	-0.04	94.88**	5.73**	5.65	0.94
i	5.31	0.68	29.74**	1.02**	-3.61	0.07	30.23**	0.01	1.58	0.43
j	-5.89	-0.38	-2.78	-0.40*	-5.31	-0.27	-2.20	0.15*	3.11	-0.02
l	-8.41	-0.32	-61.52**	-2.18**	25.12*	0.46	-60.59**	5.73**	-1.04	-0.30

NG=Number of grains, GW=Grain weight,
*Significant at 0.05, ** Significant at 0.01.

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Effects of Drought on Morphological Traits of some Sunflower Lines

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ABSTRACT

As a summer crop, sunflower is influenced a lot by environmental conditions, so sunflower yield changes frequently year by year. On the other hand, in the recent years' temperatures increased daily or periodically due to global warming. Therefore, new breeding studies should focus mostly to develop sunflower hybrids having high drought tolerance. Based on these priorities, the study was conducted to evaluate the effects of drought stress on plant height, head diameter, flowering and physiological maturity period of some male inbred lines developed previously by Trakya Agriculture Research Institute (TARI). In this study, there were big changes among male lines on tolerance levels of yield traits under controlled conditions to drought. The significant effect of drought stress was determined on head diameter among examined yield traits. However, plant height affected less from drought stress. While head diameter of plants reduced up to 50%, drought stress conditions decreased the days of flowering up to 20% (about one week) of flowering time and about 15% (about 11-12 days) of physiological maturity period. Sunflower lines were screened for improved drought tolerance based on drought factor index (DFI) calculated for head diameter values.

Keywords: Drought tolerance, Inbred lines, Sunflower, Yield traits.

Introduction

Sunflower (*Helianthus annuus* L.) is one of the most important oil crops and as a summer crop; it grows generally in rainfed regions. In these areas, drought stress is the main limiting factor for sunflower yield and other yield traits. Sunflower plants responses to drought include some processes and changes at morphological, anatomical and molecular levels mostly with decreasing photosynthesis then resulting in yield losses to adapt these stress conditions (Maury *et al*., 1996; 2000; Richard, 1996; 2006; Ali *et al*., 2003; De la Vega *et al*., 2007; Akbari *et al*., 2008; Rauf, 2008; Rauf *et al*., 2009; Hussain *et al*., 2012; Kaya *et al*., 2012; Skoric, 2012; Kaya 2014).

Drought tolerance and seed yield of cultivars are quantitative characters and are strongly influenced by environmental conditions. Therefore, their heritability is complex and these traits are determined largely by G x E conditions in addition to genetic contribution (Yordanov *et al*., 2000; Ali *et al*., 2003; De la Vega *et al*., 2007; Akbari *et al*., 2008; Haddadi *et al*. 2010; Tardieu and Tuberosa, 2010). The effects of drought stress on sunflower yield traits has been evaluated in many studies such as plant height, biological yield, stem diameter, head size, seed number per head, number of leaves per plant, 1000 seed weight, physiological maturity, harvest index, etc. (Razi and Asad, 1998; Yordanov *et al*., 2000; Poormohammad Kiani *et al*., 2007; Nezami *et al*., 2008; Petcu *et al*., 2008; Haddadi *et al*. 2010; 2011; Tardieu and Tuberosa, 2010; Pourtaghi *et al*., 2011; Abdi *et al*., 2012; Boureima *et al*., 2012; Hussain *et al*., 2012; Kaya *et al*., 2012; Skoric, 2012; Kaya 2014).

Due to inadequate stress conditions in the research fields in every year, traditional breeding programs do not work efficiently for accurate selection against drought stress, so significant progress could not be

obtained easily. Drought factor index (DFI) also is one of promising indicators among drought indices and use commonly to evaluate plant stress tolerance (Boureima *et al*., 2012). However, modern breeding tools such as molecular marker technologies, QTL, *in vitro* cultures, etc. could make great contribution for drought resistance breeding and significant successful studies are performed recently (Haddadi *et al*., 2011; Abdi *et al*., 2012).

The aim of this study was to determine drought tolerance and to evaluate performance of some yield traits of sunflower male inbred lines developed in National Sunflower project conducted by TARI under controlled stress conditions in Edirne, Turkey.

Materials and Methods

The study was carried out at TARI research fields with fifty male inbred lines originated from different genetic sources in 2014 (Table 1). Trials were conducted with RCBD with one row plots and three replications. Head size (cm), plant height (cm), flowering and physiological maturity duration (days) were observed and measured. Tunca hybrid belonging Limagrain Co was used as control. In each row, there were five plants and the distance between rows was 70 cm and 30 cm within rows. Trials were planted by hand on 29 May and plants were harvested and threshed by hand on 24 September. The rainfall and humidity in 2014 was over longer year averages while average temperatures were the same (Table 2) and daily rainfalls in 2014 (Table 3) and applied irrigations during vegetation period are given Table 4. On the other hand, chemical and physical properties of soil in the experiment field are given Table 5 and 6, respectively.

Drought Factor Index of inbred lines was calculated as DFI =Log (S_3) + 2 X Log (S_2) + 4 X Log (S_1) (Boureima *et al*., 2012). Tolerance Index (TI) of sunflower genotypes at three levels were calculated as TI (%) = (Droght Stress – Control) / [1 - (Control/100)] X 100 (Glerum, 1985). Drip irrigation was applied and as covering rain shelters, drought stress conditions were set up like below in the experiments. Stress group 1, 2 and 3 were set up on 23.06.2014, 22.07.2014 and 04.08.2014, respectively. **Control:** All plant water requirement was supplied by drip irrigation (when field capacity reduced until 50%); **Stress group 1 (S_1):** When plants were 50 cm, **Stress group 2 (S_2):** at bud development, **Stress group 3 (S_3):** at the milky stage.

Results and Discussion

Many inbred female and restorer (male) lines and F_1 hybrids were developed and released both in Turkey and also in some countries by TARI in Edirne. Therefore, to determine the level of drought tolerance of inbred lines is so important to develop better and widely adapted sunflower hybrids to plant in different conditions. Based on this study, the changes were observed in four important yield traits among sunflower restorer lines against drought stress under controlled conditions.

Plant height was almost not affected from drought stress conditions in the study (Table 7 and Figure 1). However, while there were observed increase at 1st stress, there were some decreases at 2nd and 3rd stress conditions. On the other hand, head size was influenced more from drought stress and they were reduced up to 50% (Table 8 and Figure 2).

The number of 20, 9, 4, 3, 46, 34, 6 and 50 male lines at S_1; 9, 4, 16, 27, 7, 40, 50, 34, 21, 24, and 6 at S_2 and 8, 6, 9, 14, 41, 34, 50, 4, and 21 lines affected less at S_3 from drought stress based on tolerance index of sunflower genotypes. The drought stress conditions reduced flowering time until 20% (about one week). The lines numbered as 8, 22, 43 and 24 at S_1 and 8, 22, 32, 9 and 13 influenced less at S_2 conditions but it was less comparing with other yield traits Table 9 and Figure 3). On the other hand, drought stress led their physiological maturity time 11-12 days earlier in male lines (Table 10 and Figure 4). Less affected lines were 40, 39, 2, 36, 41 and 51 at S_1 and 2, 40, 28, 41 and 35 at S_2 and 2, 33, 15, 29 and 28 at S_3 conditions.

Conclusions

In conclusion, plant head size was more influenced by drought then plant height. Therefore, as a general assessment based on DFI calculated form head size; 9758R and 7820R lines might be propounded more drought tolerant than others examined lines. In this study drought tolerant male inbred lines were identified under controlled conditions so they could be used for the future breeding research to develop tolerant sunflower hybrids.

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Table 1. The evaluated sunflower male inbred lines in the study

#	Code	#	Code	#	Code	#	Code	#	Code
1	0536 R	11	8129 R	21	9759 R	31	9997-7 R	41	TT 212 R
2	01001 R	12	8165 R	22	9761 R	32	9999 R	42	TT 214 R
3	010018 R	13	8267 R	23	9786 R	33	10004-1 R	43	TT 216 R
4	25712 R	14	TT 326 R	24	9889 R	34	10004-2 R	44	TT 317 R
5	3510 R	15	9487 R	25	9947 R	35	TT 119 R	45	TT 321 R
6	62301 R	16	9702 R	26	9979 R	36	TT 135 R	46	TT 330 R
7	6973 R	17	9753-1 R	27	9987 R	37	TT 138 R	47	K9 R SN 1
8	70352 R	18	9753-2 R	28	9990 R	38	TT 199 R	48	9868 R
9	7820 R	19	9753-3 R	29	9992 R	39	TT 205 R	49	98920 R
10	7887-1 R	20	9758 R	30	9993 R	40	TT 207 R	50	CL 217 R

Table 2. Some climatic data of longer years and in 2014 during sunflower growth period

Months	Max. Average Temp. (ºC)	Average Temp. (ºC)	Min Average Temp. (ºC)	Average Humidity (%)	Rainfall (mm)
Longer Years Averages (1954-2013)					
May	24.7	18.2	11.6	64.4	52.0
June	29.1	22.5	15. 4	60.1	44.7
July	31.7	24.7	17.3	55.9	32.0
August	31.6	24.3	17.1	56.2	23.6
September	27.1	19.8	13.3	62.2	36.8
2014 year					
May	25.0	18.6	12.5	68.7	89.0
June	28.7	22.3	16.4	67.2	88.5
July	31.9	25.3	18.3	61.9	97.8
August	32.8	25.6	18.7	61.0	12.7
September	26.5	19.6	14.5	71.4	105.3

Table 3. Daily rainfalls during the study (mm)

May	Rainfall	June	Rainfall	July	Rainfall	August	Rainfall
31 May	28,0	4 June	38,7	4 July	0,9	7 August	11,2
		5 June	6,6	5 July	0,3	18 August	5,6
		6 June	2,2	16 July	39,5		
		26 June	42,2	17 July	40,1		
				20 July	3,0		

Table 4. Irrigation amounts applied in the experiment plots (mm)

Irrigation time	Irrigation amounts (mm)	Irrigation time	Irrigation amounts (mm)
10.06.2014	50 mm	10.08.2014	75 mm
25.06.2014	70 mm	18.08.2014	60 mm
10.07.2014	65 mm	28.08.2014	60 mm
25.07.2014	40 mm		

Table 5. Chemical properties of soil in the experiment field

Soil Depth (cm)	Saturation (%)	Total Salinity (%)	pH	CaCO3 (%)	P2O5 (kg/da)	K2O (kg/da)	Organic Matter (%)
0-20	45	0,06	6,82	1,0	15,7	61,0	1,16
20-40	48	0,06	6,91	1,1	17,3	64,4	1,02

Table 6. Physical properties of soil in the experiment field

Soil Depth (cm)	Soil texture			Soil Depth (cm)	Volume Weight (gr/cm³)	Field Capacity		Wilting Point		Available water holding capacity	
	% clay	% silt	% sand			%	(mm)	%	(mm)	%	(mm)
0-20	18.7	16.6	64.5	0-30	1,68	16,21	81,69	8,23	41,48	7,98	40,22
20-40	25.0	20.8	54.2	30-60	1,71	18,78	96,34	10,12	51,92	8,66	44,42
				60-90	1,58	22,84	108,26	14,23	67,45	8,61	40,81
				90-120	1,54	19,47	89,95	12,79	59,09	6,68	30,86
				0-90			286,29		160,85		125,45

Table 7. The effect of drought stress on plant heights (cm) in sunflower

#	Name of Line	Control	Stress 1	Tolerance Index (%)	Stress 2	Tolerance Index (%)	Stress 3	Tolerance Index (%)
23	9786 R	90,00	108,33	120,4	87,33	**97,0**	85,00	94,4
47	K9 R SN 1	146,00	162,67	111,4	133,67	91,6	136,67	93,6
26	9979 R	128,33	141,33	110,1	121,33	94,5	123,33	96,1
33	10004-1 R	107,33	118,00	109,9	102,00	95,0	111,00	103,4
3	010018 R	127,00	139,00	109,4	124,00	**97,6**	126,00	**99,2**
11	8129 R	131,00	143,33	109,4	127,33	**97,2**	128,33	**98,0**
18	9753-2 R	95,33	103,67	108,7	90,33	94,8	92,67	97,2
24	9889 R	117,00	127,00	108,5	107,00	91,5	112,67	96,3
38	TT 199 R	139,00	149,67	107,7	136,33	**98,1**	138,67	**99,8**
37	TT 138 R	112,00	120,67	107,7	99,67	89,0	101,33	90,5
29	9992 R	124,00	131,67	106,2	114,00	91,9	119,33	96,2
48	9868 R	156,33	165,00	105,5	145,33	93,0	142,00	90,8
34	10004-2 R	96,67	101,33	104,8	91,00	94,1	95,00	**98,3**
19	9753-3 R	96,33	101,00	104,8	84,67	87,9	87,33	90,7
16	9702 R	105,00	109,67	104,4	91,33	87,0	95,67	91,1
4	25712 R	115,67	120,67	104,3	122,00	105,5	120,67	104,3
21	9759 R	133,00	138,33	104,0	132,33	**99,5**	132,00	**99,2**
27	9987 R	127,00	132,00	103,9	124,67	**98,2**	121,33	95,5
39	TT 205 R	120,00	124,67	103,9	105,67	88,1	111,00	92,5
51	Tunca (S)	175,67	182,00	103,6	164,33	93,5	167,33	95,3
17	9753-1 R	100,00	103,00	103,0	97,67	**97,7**	93,33	93,3
2	01001 R	124,33	127,33	102,4	123,33	**99,2**	120,33	96,8
10	7887-1 R	128,33	131,33	102,3	122,00	95,1	122,33	95,3
5	3510 R	140,67	143,67	102,1	135,00	96,0	134,00	95,3
31	9997-7 R	144,67	147,00	101,6	127,00	87,8	132,33	91,5

Continuing table 7

#	Name of Line	Control	Stress 1	Tolerance Index (%)	Stress 2	Tolerance Index (%)	Stress 3	Tolerance Index (%)
46	TT 330 R	112,33	113,67	**101,2**	108,33	**96,4**	111,33	**99,1**
32	9999 R	110,67	112,00	**101,2**	107,00	**96,7**	108,67	**98,2**
15	9487 R	126,00	127,33	**101,1**	117,00	92,9	119,67	95,0
43	TT 216 R	137,67	138,67	**100,7**	125,33	91,0	130,33	94,7
14	TT 326 R	127,00	127,67	**100,5**	120,33	94,8	119,00	93,7
35	TT 119 R	141,33	142,00	**100,5**	129,33	91,5	132,00	93,4
36	TT 135 R	133,33	133,33	**100,0**	125,67	94,3	130,67	**98,0**
28	9990 R	158,00	157,33	**99,6**	147,67	93,5	145,00	91,8
6	62301 R	158,33	157,00	**99,2**	150,67	95,2	147,33	93,1
25	9947 R	138,33	136,67	**98,8**	132,67	95,9	136,00	**98,3**
44	TT 317 R	161,00	159,00	**98,8**	143,33	89,0	147,33	91,5
41	TT 212 R	142,00	139,67	**98,4**	127,33	89,7	129,67	91,3
8	70352 R	122,00	119,00	97,5	114,33	93,7	115,67	94,8
40	TT 207 R	149,00	145,33	97,5	128,00	85,9	135,67	91,1
22	9761 R	117,67	114,33	97,2	110,33	93,8	114,67	97,5
12	8165 R	148,00	143,67	97,1	137,33	92,8	131,00	88,5
42	TT 214 R	132,00	128,00	97,0	114,33	86,6	112,00	84,8
45	TT 321 R	115,00	111,33	96,8	108,67	94,5	106,00	92,2
20	9758 R	170,33	163,00	95,7	160,33	94,1	157,67	92,6
9	7820 R	127,67	122,00	95,6	123,00	96,3	123,33	96,6
1	0536 R	123,33	116,00	94,1	128,33	**104,1**	124,00	**100,5**
30	9993 R	129,33	120,00	92,8	125,00	**96,6**	122,33	94,6
7	6973 R	141,00	126,00	89,4	121,33	86,1	126,00	89,4
50	CL 217 R	130,67	113,67	87,0	119,33	91,3	121,00	92,6
$\bar{x}$: LSD 0,01):1,81		130,3 B	132,2 A		121,9 C		123,2 C	

Table 8. The effect of drought stress on head size (cm) in sunflower

#	Name of Line	Control	Stress 1	Tolerance Index (%)	Stress 2	Tolerance Index (%)	Stress 3	Tolerance Index (%)
20	9758 R	10,00	10,67	**106,7**	8,00	80,0	8,00	80,0
9	7820 R	13,00	11,33	**87,2**	12,00	**92,3**	12,33	**94,9**
4	25712 R	11,67	9,00	**77,1**	10,67	**91,4**	10,67	**91,4**
3	010018 R	14,33	11,00	**76,7**	12,33	86,0	13,00	**90,7**
46	TT 330 R	14,00	10,67	**76,2**	11,33	81,0	11,67	83,3
34	10004-2 R	12,33	9,33	**75,7**	11,00	**89,2**	11,33	**91,9**
6	62301 R	13,67	10,33	**75,6**	12,00	**87,8**	13,00	**95,1**
50	CL 217 R	16,00	12,00	**75,0**	14,33	**89,6**	14,67	**91,7**
25	9947 R	14,67	11,00	**75,0**	12,00	81,8	12,33	84,1
33	10004-1 R	14,00	10,33	73,8	11,33	81,0	12,00	85,7
38	TT 199 R	13,33	9,67	72,5	11,67	**87,5**	11,67	87,5
24	9889 R	14,33	10,33	72,1	12,67	**88,4**	13,00	**90,7**
41	TT 212 R	19,00	13,67	71,9	16,33	86,0	17,67	**93,0**
8	70352 R	11,67	8,33	71,4	10,00	85,7	11,67	**100,0**
42	TT 214 R	16,33	11,67	71,4	14,00	85,7	13,67	83,7
11	8129 R	11,67	8,33	71,4	9,67	82,9	10,00	85,7
39	TT 205 R	14,00	10,00	71,4	11,00	78,6	11,67	83,3
49	98920 R	16,00	11,33	70,8	12,67	79,2	13,67	85,4
7	6973 R	13,67	9,67	70,7	12,33	**90,2**	12,00	87,8
1	0536 R	13,67	9,67	70,7	11,33	82,9	12,33	**90,2**
21	9759 R	14,67	10,33	70,5	13,00	**88,6**	13,33	**90,9**
26	9979 R	14,67	10,33	70,5	12,67	86,4	13,33	**90,9**
35	TT 119 R	14,67	10,33	70,5	12,00	81,8	12,33	84,1
27	9987 R	19,00	13,33	70,2	17,33	**91,2**	17,00	89,5
12	8165 R	15,33	10,67	69,6	13,00	84,8	12,67	82,6

Continuing table 8

#	Name of Line	Control	Stress 1	Tolerance Index (%)	Stress 2	Tolerance Index (%)	Stress 3	Tolerance Index (%)
31	9997-7 R	14,67	10,00	68,2	11,33	77,3	12,00	81,8
36	TT 135 R	13,33	9,00	67,5	11,00	82,5	11,33	85,0
51	Tunca (S)	20,00	13,33	66,7	17,33	86,7	18,00	90,0
13	8267 R	14,00	9,33	66,7	10,67	76,2	11,67	83,3
32	9999 R	11,00	7,33	66,7	8,00	72,7	8,67	78,8
23	9786 R	15,67	10,33	66,0	13,67	**87,2**	13,00	83,0
43	TT 216 R	15,67	10,33	66,0	13,33	85,1	14,00	89,4
17	9753-1 R	14,67	9,67	65,9	12,67	86,4	12,67	86,4
30	9993 R	11,67	7,67	65,7	9,67	82,9	10,33	88,6
16	9702 R	15,33	10,00	65,2	14,00	**91,3**	13,67	89,1
14	TT 326 R	14,33	9,33	65,1	11,33	79,1	13,33	**93,0**
10	7887-1 R	10,33	6,67	64,5	8,67	83,9	9,00	87,1
2	01001 R	14,67	9,33	63,6	11,00	75,0	12,67	86,4
37	TT 138 R	13,67	8,67	63,4	11,00	80,5	10,67	78,0
19	9753-3 R	14,33	9,00	62,8	11,67	81,4	12,33	86,0
40	TT 207 R	19,67	12,33	62,7	17,67	**89,8**	17,33	88,1
47	K9 R SN 1	19,33	12,00	62,1	15,33	79,3	16,00	82,8
44	TT 317 R	20,33	12,33	60,7	16,67	82,0	16,33	80,3
5	3510 R	9,33	5,67	60,7	6,67	71,4	7,33	78,6
48	9868 R	14,67	8,67	59,1	11,33	77,3	12,00	81,8
28	9990 R	13,00	7,67	59,0	10,33	79,5	11,33	87,2
15	9487 R	15,33	8,67	56,5	11,67	76,1	12,00	78,3
18	9753-2 R	16,67	8,67	52,0	14,00	84,0	14,00	84,0
22	9761 R	17,33	8,33	48,1	13,33	76,9	14,33	82,7
$\bar{x}$: LSD (0,01):0,3		14,57 A	9,94 D		12,15 C		12,62 B	

Table 9. The effect of drought stress on flowering period (day) in sunflower

#	Name of Line	Control	Stress 1	Tolerance Index (%)	Stress 2	Tolerance Index (%)
8	70352 R	55,3	53,7	**97,0**	55,3	**100,0**
22	9761 R	55,7	54,0	**97,0**	55,3	**99,4**
43	TT 216 R	58,7	56,7	**96,6**	57,7	98,3
24	9889 R	60,3	58,0	**96,1**	59,3	98,3
16	9702 R	60,0	57,7	**96,1**	58,7	97,8
9	7820 R	59,0	56,7	**96,0**	58,3	**98,9**
13	8267 R	55,7	53,3	95,8	55,0	**98,8**
32	9999 R	58,7	56,0	95,5	58,3	**99,4**
10	7887-1 R	57,3	54,3	94,8	56,3	98,3
27	9987 R	56,7	53,7	94,7	54,7	96,5
36	TT 135 R	54,7	51,7	94,5	54,0	**98,8**
51	Tunca (S)	60,3	57,0	94,5	58,3	96,7
35	TT 119 R	59,7	56,3	94,4	58,0	97,2
49	98920 R	63,7	60,0	94,2	61,3	96,3
11	8129 R	61,3	57,7	94,0	60,0	97,8
44	TT 317 R	65,7	61,7	93,9	64,0	97,5
18	9753-2 R	53,7	50,3	93,8	52,7	98,1
33	10004-1 R	57,3	53,7	93,6	56,3	98,3
12	8165 R	61,0	57,0	93,4	58,7	96,2
50	CL 217 R	64,7	60,3	93,3	62,0	95,9
17	9753-1 R	53,7	50,0	93,2	53,0	**98,8**
29	9992 R	57,3	53,3	93,0	55,3	96,5
37	TT 138 R	56,0	52,0	92,9	54,7	97,6
19	9753-3 R	54,3	50,3	92,6	53,0	97,5
38	TT 199 R	56,3	52,0	92,3	54,7	97,0

Continuing table 9

#	Name of Line	Control	Stress 1	Tolerance Index (%)	Stress 2	Tolerance Index (%)
40	TT 207 R	59,0	54,3	92,1	56,7	96,0
31	9997-7 R	60,7	55,7	91,8	58,7	96,7
15	9487 R	61,0	56,0	91,8	58,3	95,6
14	TT 326 R	60,7	55,7	91,8	57,7	95,1
42	TT 214 R	60,3	55,3	91,7	58,3	96,7
7	6973 R	59,3	54,3	91,6	57,7	97,2
46	TT 330 R	59,7	54,7	91,6	58,0	97,2
30	9993 R	63,0	57,7	91,5	60,3	95,8
3	010018 R	57,7	52,7	91,3	55,3	96,0
1	0536 R	64,3	58,7	91,2	62,0	96,4
21	9759 R	59,3	54,0	91,0	57,3	96,6
41	TT 212 R	58,7	53,3	90,9	57,7	98,3
39	TT 205 R	56,7	51,3	90,6	54,3	95,9
47	K9 R SN 1	59,7	54,0	90,5	57,0	95,5
6	62301 R	65,3	59,0	90,3	61,3	93,9
2	01001 R	64,3	58,0	90,2	60,7	94,3
48	9868 R	61,0	55,0	90,2	57,3	94,0
34	10004-2 R	61,0	54,7	89,6	58,3	95,6
28	9990 R	60,7	53,7	88,5	56,7	93,4
4	25712 R	61,0	54,0	88,5	56,0	91,8
45	TT 321 R	61,3	54,0	88,0	57,3	93,5
25	9947 R	63,3	55,7	87,9	58,3	92,1
26	9979 R	64,0	56,0	87,5	59,3	92,7
23	9786 R	61,7	53,7	87,0	58,0	94,1
5	3510 R	61,7	52,3	84,9	55,0	89,2
$\bar{x}$: LSD (0,01):0,66		59,7 A	55,1 C		57,5 B	

Table 10. The effect of drought stress on physiological maturity period (day) in sunflower

#	Name of Line	Control	Stress 1	Tolerance Index (%)	Stress 2	Tolerance Index (%)	Stress 3	Tolerance Index (%)
40	TT 207 R	91,7	86,0	93,8	88,3	96,4	89,7	97,8
39	TT 205 R	92,7	86,0	92,8	86,7	93,5	90,3	97,5
2	01001 R	96,7	89,3	92,4	94,3	97,6	97,0	100,3
36	TT 135 R	87,7	80,3	91,6	84,0	95,8	85,7	97,7
41	TT 212 R	94,3	86,3	91,5	90,7	96,1	91,0	96,5
51	Tunca (S)	101,0	92,3	91,4	93,0	92,1	98,3	97,4
28	9990 R	95,0	86,7	91,2	91,3	96,1	93,7	98,6
38	TT 199 R	91,3	83,0	90,9	86,3	94,5	89,7	98,2
37	TT 138 R	91,7	83,3	90,9	86,7	94,5	89,7	97,8
29	9992 R	90,7	82,3	90,8	86,3	95,2	89,7	98,9
11	8129 R	94,0	85,3	90,8	87,7	93,3	90,0	95,7
43	TT 216 R	93,3	84,7	90,7	89,0	95,4	91,0	97,5
26	9979 R	99,7	90,3	90,6	94,7	95,0	98,0	98,3
35	TT 119 R	92,3	83,3	90,3	88,7	96,0	91,0	98,6
22	9761 R	91,7	82,7	90,2	86,3	94,2	89,3	97,5
48	9868 R	102,0	91,3	89,5	95,3	93,5	98,7	96,7
3	010018 R	91,7	82,0	89,5	85,3	93,1	88,7	96,7
4	25712 R	94,3	84,3	89,4	89,3	94,7	91,0	96,5
49	98920 R	99,3	88,7	89,3	93,7	94,3	96,3	97,0
24	9889 R	93,7	83,7	89,3	87,3	93,2	92,0	98,2
46	TT 330 R	93,7	83,7	89,3	87,0	92,9	90,3	96,4
5	3510 R	95,0	84,7	89,1	89,7	94,4	92,3	97,2
8	70352 R	89,0	79,3	89,1	83,3	93,6	84,7	95,1
50	CL 217 R	99,3	88,3	88,9	94,3	95,0	97,7	98,3

Continuing table 10

#	Name of Line	Control	Stress 1	Tolerance Index (%)	Stress 2	Tolerance Index (%)	Stress 3	Tolerance Index (%)
30	9993 R	96,3	85,7	88,9	89,3	92,7	93,3	96,9
32	9999 R	92,7	82,3	88,8	88,7	95,7	90,3	97,5
16	9702 R	95,3	84,7	88,8	88,7	93,0	92,0	96,5
42	TT 214 R	95,3	84,7	88,8	88,0	92,3	92,3	96,9
23	9786 R	96,7	85,7	88,6	92,3	95,5	94,3	97,6
15	9487 R	92,7	82,0	88,5	86,0	92,8	91,7	98,9
19	9753-3 R	85,7	75,7	88,3	81,3	**94,9**	83,7	97,7
9	7820 R	94,3	83,3	88,3	86,7	91,9	91,3	96,8
47	K9 R SN 1	95,7	84,3	88,2	91,7	95,8	93,7	97,9
20	9758 R	99,7	87,7	88,0	89,3	89,6	96,7	97,0
31	9997-7 R	95,7	84,0	87,8	88,3	**92,3**	94,3	98,6
10	7887-1 R	92,3	80,3	87,0	83,7	90,6	87,3	**94,6**
21	9759 R	94,7	82,3	87,0	84,3	89,1	91,0	96,1
12	8165 R	94,0	81,7	86,9	86,7	92,2	91,0	96,8
7	6973 R	94,3	81,7	86,6	84,0	89,0	88,3	93,6
13	8267 R	93,3	80,7	86,4	83,7	89,6	90,0	96,4
14	TT 326 R	95,3	82,3	86,4	85,0	**89,2**	90,7	95,1
45	TT 321 R	96,0	82,7	86,1	90,3	94,1	93,3	97,2
18	9753-2 R	86,3	74,0	85,7	79,7	92,3	84,7	98,1
44	TT 317 R	101,3	86,3	85,2	90,3	89,1	97,3	96,1
25	9947 R	96,7	81,7	84,5	85,7	88,6	88,7	91,7
34	10004-2 R	96,3	78,7	81,7	85,3	88,6	90,7	94,1
33	10004-1 R	94,3	74,3	78,8	90,3	95,8	93,7	99,3
$\bar{x}$: LSD (0,01):0,45		94,4 A	83,6 D		88,1 C		91,6 B	

Figure 1. DFI Index on plant heights of sunflower lines

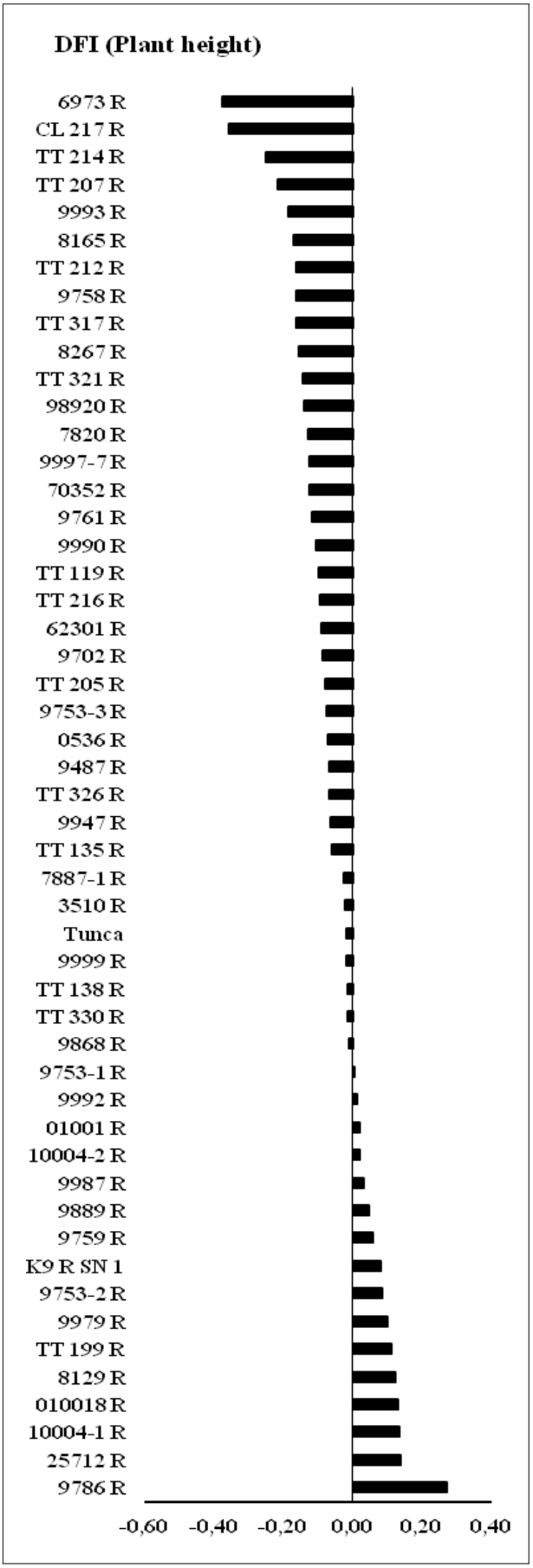


Figure 2. DFI Index on head diameters of sunflower lines

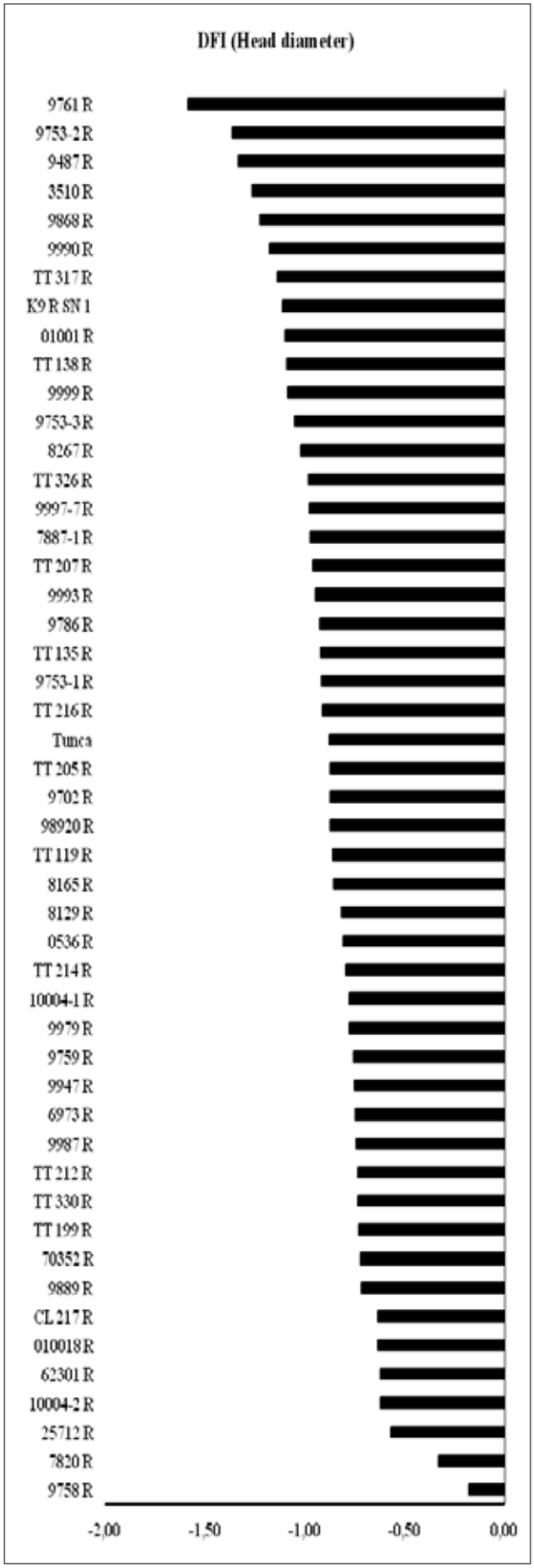


Figure 3. DFI Index on flowering time of sunflower lines

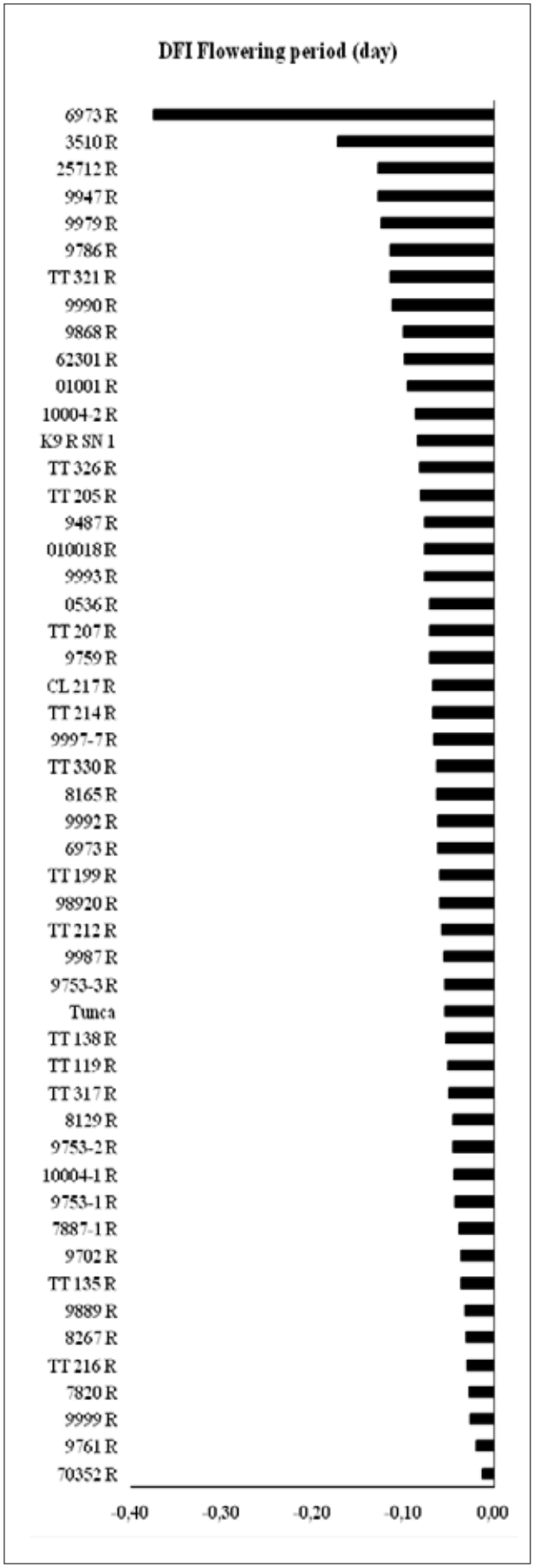
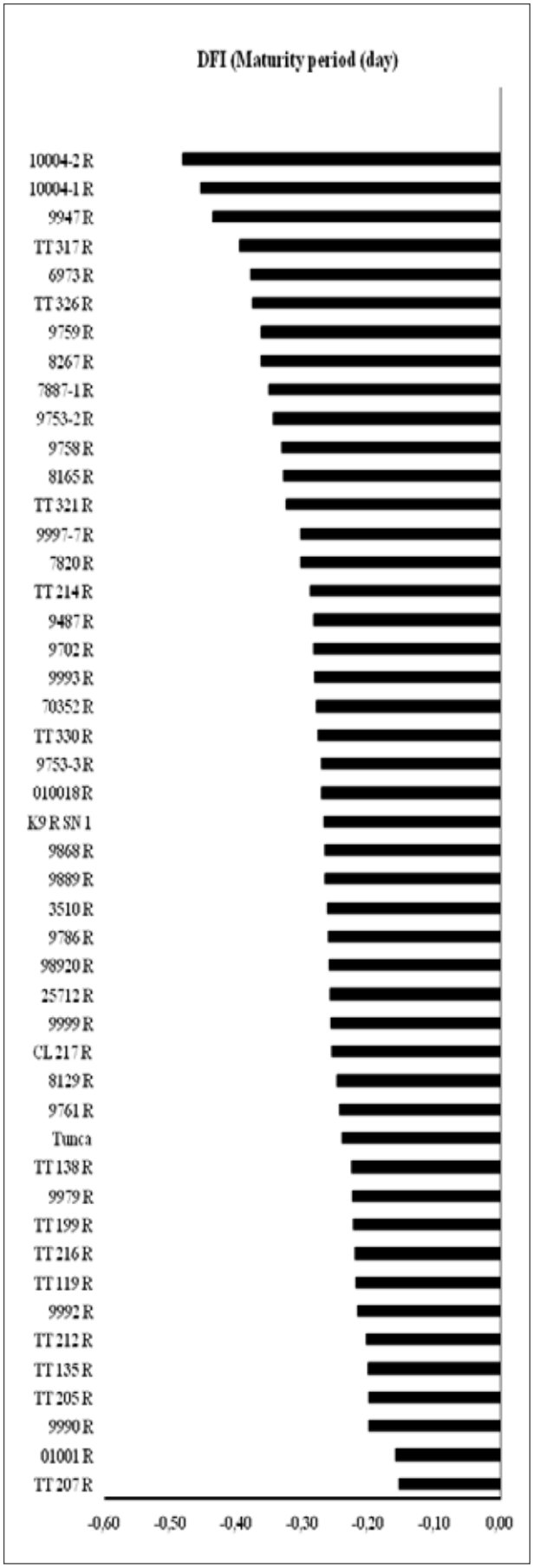


Figure 4. DFI Index on physiological maturity of sunflower lines



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The Variation of Crude Protein and Total Fat of the Main Grassland Plants, in Various Stages of Growth, in "Kostilata" Subalpine Grassland in Theodoriana, Arta, Greece

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ABSTRACT

The chemical composition of different plant parts varies, both within the plant itself, as well as among different plants, which is due to their structure, stage of growth and phenological characteristics. In this study, the results of a research conducted in 2013, in a subalpine ecosystem in Epirus (Greece) are presented. The objectives of this research were the determination of crude protein and total fat (ether extract) of the ecosystem's main grassland plants, per group (grasses, legumes, other forbs), at different stages of growth. These plant species were: a) grasses (*Alopecurus gerardil* Vill, *Stipa pennata* L., *Phleum alpinum* L.), b) legumes (*Trifolium repens* L, *Lathyrus aphaca* L, *Lotus corniculatus* L.) and c) other forbs (*Ranunculus repens* L., *Achillea millefolium* L. and *Geranium lucidum* L.). The results showed that: a) the largest amount in crude protein was found in *Trifolium repens* L. with a percentage of 17.05%, with a statistically significant difference only with grasses, b) in all plant species, the largest amounts of crude protein and total fat was observed during the initial stage of plant growth, showing a gradual decrease until the final stage of growth, c) statistically significant differences appeared in the amount of crude protein and total fat, between the same plant species, at different sampling dates, and between different plant species in the same sampling dates and d) the amount of total fat in all plant species was on average 3%.

Keywords: Subalpine grassland, crude protein, total fat.

Introduction

The subalpine grasslands have a rich flora and are used primarily by pastoralism. Animal nutrition is the main factor that determines the quality of animal products (Boyazoglu and Morand-Fehr, 2001; Coulon *et al*., 2004). It is well known that ruminant animals cover a large part of their dietary needs, by grazing, which ranges from 25% to 75% (Zervas, 1998), while, animal nutrition represents 50% to 90% of the production costs of each animal product (Ruiz *et al*., 2009). Finally, rearing systems which are based on grazing, give animal products of high nutritional value (Buchin *et al.*, 1999; Viallon *et al.*, 2000; Noziere *et al.*, 2006).

The amount of crude protein in grassland plants is one of the most important factors that define the quality of the produced forage (Buxton, 1996; Bell, 2003; Mlay *et al.,* 2006). At a specific time, the stage of plant growth varies between different plant species (Tallowin and Jefferson, 1999; Bruinenberg *et al.*, 2002) and the main factors that affect the growth of plants in natural conditions are precipitation and air temperature (Frank and Ries, 1990; Papanastasis *et al*., 1997;

Tallowin and Jefferson, 1999; Lemaire *et al.*, 2000). The amount of crude protein in grassland plants, at an early stage of growth is higher than that of plants at a mature stage of growth (Buxton, 1996; Minson, 1990; Tzialla *et al.* 2000; Ammar *et al.*, 2004; Duru and Ducrocq, 1997; Hejcman *et al.*, 2010; Mountousis, 2008; Roukos *et al.*, 2006; Perez Corona *et al.*, 1998). More specifically, plant leaves contain higher percentage of crude protein than the stems and shoots, even at higher stage of growth (Cook, 1972; Ganskopp and Bohnert, 2001). As the plants grow, the ratio of leaf and stem usually decreases (Albrecht *et al.*, 1987; Buxton, 1996). The amount of crude protein in legumes is higher than that of grasses (Minson, 1990), while the amount of crude protein in forbs lies between that of legumes and grasses (Cook, 1972; Krysl *et al.*, 1984; Meyer and Brown, 1985; Ruyle, 1993).

The total fat or in other words, ether extract comprises the group of nutrient fat, which plays very important role in the animal body (Liamadis, 2000) and it is a very important energy component of the ruminant feed (Bauman *et al*., 2003). The amount of fat in forage is, generally, low (less than 3% of the dry matter) (Coleman and Henry, 2002; Bruinenberg, 2003). The amount of total fat in plants decreases with growth (SCA, 1990), while the leaves of the plants contain higher amount of fat than the stems (Cook, 1972). The milk fat and the rate and type of fatty acids are affected by the rate of feed coming from grazing (Avondo *et al.*, 2003; Nudda *et al.*, 2003), as well as the plant species and their phenological stage (Addis *et al.*, 2005; Cabiddu *et al.*, 2005).

The use of the grasslands by extensive livestock farming, contributes to the production of quality animal products, the preservation of biodiversity and the protection of ecosystems themselves from natural hazards (Hadjigeorgiou *et al*., 2005; Chatzitheodoridis *et al.*, 2007). Also, the rational use of grasslands requires both the knowledge of the nutritional needs of animals, as well as the quantity and quality of rangeland production in specific soil and climatic environments (Holechek *et al.*, 1995).

The "Kostilata" subalpine grassland is used only by pastoralism, it is of low production, dominated by grasses and it needs to be rationally managed (Roukos *et al.*, 2014). In this study, the variation in chemical composition (crude protein, total fat) of the main plants, per group (grasses, legumes, other forbs), in different stages of growth is described.

Materials and Methods

The research was conducted in 2013, in "Kostilata" subalpine grassland, and it extends at an altitude of 1400 to 2393 m., it is located, approximately, 80 km northeast of Arta, in Theodoriana, in the mountain range of Tzoumerka. Sixty (60) fixed experimental cages, one meter high, made of mesh, with dimensions 4 m x 4 m, were installed, to protect plants from grazing. The cages were placed, randomly, in such a way so as to be representative of the grassland's vegetation. The aboveground biomass was collected, with the aid of a metallic frame, with dimensions of 50 x 50 cm, from five (5) different positions, within each of the cages, in order to have homogeneity, according to the method of harvesting (Odum; 1971, Cook and Stubbendieck, 1986; Sarlis, 1998). The samplings were carried out from April 30th to July 15th and specifically at 30/4, 16/5 8/6, 17/6 28/6 and 15/7, during which the animals were grazing. Forage was separated, from each sample, in three main groups: grasses, legumes and other forbs. From each group, three plant species, which had the highest proportion of biomass were selected: a) from grasses (*Alopecurus gerardil* Vill, S*tipa pennata* L., *Phleum alpinum* L.), b) from legumes (*Trifolium repens* L, *Lathyrus aphaca* L, *Lotus corniculatus* L. and c) from other forbs *(Ranunculus repens* L.*, Achillea millefolium* L. and *Geranium lucidum* L. For the determination of the plant species the encyclopedia "Mountain Flora of Greece I and II" (Strid, 1986, Strid and Tan, 1991), the book "The main grasses of natural grasslands" (Papanastasis *et al*., 1993) and the book "Vascular Plants of Greece" (Dimopoulos *et al*., 2013) were used, whereas, for receiving the climate parameters (air temperature and precipitation), the weather station which is installed in Theodoriana, was used (Table 1). Likewise characteristics of soil of study area are given in Table 2.Then, the samples were placed in an oven for drying, at 65°C for 48 hours (Deinum and Maassen, 1994). The determination of crude protein was made according to the Kjeldahl method (A.O.A.C., 1999), while for the determination of total fat, an extraction of the samples was made, in petroleum ether, by using the Soxherm apparatus, according to the Soxhlet method (A.O.A.C., 1990).

The results were compared for significant differences by one-way ANOVA test while mean differences were checked using Tuckey's test ($p<0.05$). Statistical analyses were performed also with OriginPro 9.0 software.

Results and Discussion

Crude Protein

The largest amount in crude protein, on average, was found in *Trifolium repens* L. at a rate of 17.05%, in *Lotus corniculatus* L. at a rate of 14.86% and in *Lathyrus aphaca* L. at a rate of 14.76%. The other forbs followed and especially, *Ranunculus repens* L. with 13.88%, *Achillea millefolium* L., with 12.52% and *Geranium lucidum* L. with 11.80%, while, grasses showed the lowest percentage rates, 9.68%, 9.34%

and 10.79%, in *Alopecurus gerardii* Vill, in *Stipa pennata* L. and in *Phleum alpinum* L. respectively, with a statistically significant difference to be observed only between *Trifolium repens* L. and grasses (Table 3). Legumes contain larger quantity of crude protein compared to other plants (Minson, 1990; Ruyle, 1993). The results of our research agree with those of Minson (1990), who found that legumes contain crude proteins at a rate of around 16% to 17%, of the dry matter, while grasses contain crude proteins, at a rate of around 10% to 13%. Also, the rates of crude protein recorded by Meyer and Brown, (1985) and Ruyle, (1993) in other forbs, were lying between those of grasses and legumes.

The higher rates in all plant species occurred, with a statistically significant difference, in their early growth stages and decreased as the plants matured. The decrease in the amount of crude protein in plants is due to the fact that leaves have a higher content of protein, compared to the stems (Ganskopp and Bohnert, 2001), whereas, as the plants mature, the ratio of leaf / stem, usually decreases (Albrecht *et al*., 1987; Buxton, 1996). Also, the decrease of crude protein in plants, during plant growth, has been reported by Duru and Ducrocq, (1997) and Hejcman *et al*., (2010).

Also, a statistically significant difference was observed between plant species, on the same sampling dates, mainly, between both legumes and other forbs and grasses. These differences are due to the fact that, at a specific time, the stages of growth vary between different plant species (Tallowin and Jefferson, 1999; Bruinenberg *et al*. 2002).

Total fat

The largest amount of total fat, on average, was observed in *Lathyrus aphaca* L. with 2.90%, while the other forbs and grass showed lower rates. Statistically, significant difference was observed both between the different growth stages of the same plant, as well as between different plant species at the same sampling dates, mainly between both legumes and other forbs and grasses. Also, the higher rates in all plant species occurred, with a statistically significant difference, in their early growth stages and decreased as the plants matured (Table 4). All the above are due to the fact that leaves have a higher content of total fat, compared to the stems (Cook, 1972) and that the content of the plants in total fat decreases as the plants mature (SCA, 1990), while, at a specific time, the stages of growth vary between different plant species (Tallowin and Jefferson, 1999; Bruinenberg *et al*., 2002).

Also, according to Albrecht *et al*., (1987) and Buxton, (1996), as the plants mature, the ratio of leaf / stem, usually decreases. The results of our research agree with those of Coleman and Henry, (2002) and Bruinenberg, (2003), who report that the content of forage dry matter, in total fat is less than 3%.

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Table 1. Climatic data of the region, the years 2010, 2011, 2012 and 2013 (National Meteorological Station, 2014)

Year	Average, annual air temperature (^{0}C)	Average, annual precipitation (mm)
2010	11.27	2.887
2011	10.66	1.549,1
2012	11.53	3.240,8
2013	11.55	3.143,4
Mean	11.25	2.705,2

Table 2. Characteristics of the soil of the study area (Roukos *et al*., 2014)

Parameter	Clay	Slit	Sand	pH	Organic matter	$CaCO_3$	P
	(%)	(%)	(%)		(%)	g / kg	g / kg
Mean	14.5	36.0	49.5	5.6	6.8	0.465	14.5
Typ. error	0.72	0.77	1.05	0.06	0.24	0.39	2.60

Table 3. Content of crude protein in plants, at various stages of growth, on average.

Group	Species	Sampling dates of the year 2013							
		30/4	16/5	27/5	8/6	17/6	28/6	15/7	Average
Grasses	*Alopecurus gerardii* L.	.....	13.27 ± 3.15a^{14}	10.47 ± 0.53ab^{1}	11.06 ± 0.46ab^{13}	7.93 ± 0.74 bc^{1}	8.36 ± 0.12 bc^{1}	6.96 ± 0.63bc^{1}	9.68 ± 0.62^{1}
	Stipa pennata L.	.....	11.97 ± 0.86a^{1}	10.78 ± 0.04ab^{1}	9.88 ± 0.05ab^{1}	8.88 ±1.46abc^{1}	8.38 ± 0.39bc^{1}	6.16 ± 0.33c^{1}	9.34 ± 0.46^{1}
	Phleum alpinum L.	.....	.....	.....	11.52 ± 1.27a^{1}	12.36 ± 2.21a ^{1}	10.71 ± 1.09a^{13}	8.57 ± 0.56a^{1}	10.79 ± 1.29^{1}
Legumes	*Trifolium repens* L	.....	18.91 ± 1.60a ^{2}	21.43 ± 0.68a ^{2}	18.53 ± 0.48a^{2}	19.62 ± 0.80 a^{2}	12.45 ± 0.37b^{2}	11.33 ± 0.60b^{2}	17.05 ± 0.8^{12}
	Lathyrus aphaca L.	.....	13.50 ± 0.48ab^{1}	17.50 ± 3.03a^{23}	19.29 ± 1.59 a^{2}	17.34 ± 0.32a^{2}	14.11 ±1.66ab^{2}	6.8 ± 0.16b^{2}	14.76 ±1.21^{12}
	Lotus corniculatus L.	.....	16.68 ± 0.58a^{24}	16.02 ± 0.26a^{3}	18.87 ± 0.48a^{2}	17.12 ± 1.31a^{2}	11.74 ± 0.32 b^{23}	8.74 ± 0.11b^{1}	14.86 ± 0.68^{12}
Other forbs	*Ranunculus repens* L.	19.90 ± 0.84a	15.63 ±1.36b^{234}	14.13 ± 0.21b^{34}	13.11 ± 0.46b^{13}	11.85 ± 0.25bc^{13}	8.65 ± 0.49c^{1}	.....	13.88 ± 0.60^{12}
	Achillea millefolium L.	.....	13.68 ± 1.95a^{1}	14.81 ±1.78a^{34}	13.82 ± 0.37a^{13}	12.39±1.09a^{3}	12.17 ± 1.33a^{2}	8.23 ± 0.17b^{1}	12.52 ± 1.11^{12}
	Geranium lucidum L.	.....	18.35 ± 0.21a^{23}	15.81 ± 0.38b^{34}	9.49 ± 0.19c^{1}	10.51±0.38c^{13}	9.65 ± 0.37c^{1}	6.98 ± 0.12d^{1}	11.80 ± 0.28^{12}
	Mean	19.9 ± 0.84a	15.20 ± 0.80a	15.21 ± 0.96a	15.98 ± 0.70a	14.58 ± 0.85a	10.76 ± 0.53b	8.97 ± 0.54b	

* Means with different superscripts (a ,b, c, d) in each row, differ significantly (P < 0,05)

** Means with different exponent (1 2 3 4) in each column differ significantly (P < 0,05)

Table 4. Content of total fat in plants, at various stages of growth, on average.

Group	Species	Sampling dates of the year 2013							
		30/4	16/5	27/5	8/6	17/6	28/6	15/7	Average
Grasses	*Alopecurus gerardii* L.	……	2.95 ± 0.29a[1]	1.78 ± 0.23 ab[1]	1.50 ± 0.11b[1]	0.86 ± 0.09b[1]	1.09 ± 0.49b 1	1.73 ± 0.13b[1]	1.65 ± 0.73[1]
	Stipa pennata L.	……	3.04 ± 0.02a[1]	1.92 ± 0.03b[1]	1.88 ± 0.09bc[2]	2.76 ± 0.05ad[2]	1.94 ± 0.26bcd[12]	2.09 ±0.23bcd[1]	2.27 ± 0.50[1]
	Phleum alpinum L.	……	……..	…….	2.13 ± 0.37a[2]	2.65 ± 0.36[2]	1.55 ± 0.22b[13]	1.29 ± 0.18bc[1]	1.90 ± 0.60[1]
Legumes	*Trifolium repens* L	……	3.24 ± 0.47 a[1]	2.57 ± 0.22ab[2]	1.74 ± 0.19b[21]	1.86 ± 0.12b[34]	1.83 ± 0.23b[13]	1.44 ± 0.42b[1]	2.11 ± 0.66[1]
	Lathyrus aphaca L.	……	4.0 ± 0.20a[1]	2.97 ± 0.14ac[2]	2.18 ± 0.22cb[2]	2.51± 0.31abc[2]	2.15 ± 0.14bc[23]	3.62 ± 0.60a[2]	2.90 ± 0.77[1]
	Lotus corniculatus L.	……	3.80 ± 0.41a[1]	3.88 ± 0.56a[2]	3.40 ± 0.86a[3]	3.40 ± 0.86b[2]	1.97 ± 0.16 b[12]	1.43 ± 0.32c[1]	2.7 ± 1.04[1]
Other forbs	*Ranunculus repens* L.	3.30 ± 0.15a	2.58 ±0.29ab[1]	2.81 ± 0.31a[2]	2.41 ± 0.10ab[2]	1.63 ± 0.21b[3]	1.51 ± 0.04b[1]	……	2.37 ± 0.69[1]
	Achillea millefolium L.	……	2.74 ± 0.76a[1]	3.07 ± 0.44a[2]	1.85 ± 0.01a[2]	1.81 ± 0.03a[3]	2.8 ± 0.52a[234]	2.32 ± 0.12a[1]	2.43 ± 0.52[1]
	Geranium lucidum L.	……	3.67±0.14a[1]		2.08 ± 0.31bc[2]	2.02 ± 0.33bc[34]	3.03 ± 0.19a[4]	1.64 ± 0.14dc[1]	2.58 ± 0.79[1]
	Mean	3.30 ±0.15a	3.09 ± 0.35 a	2.75 ± 0.29a	2.13 ± 0.25a	2.03 ± 0.17a	2.03 ± 0.26b	1.94 ± 0.22b	

* Means with different superscripts (a, b, c, d) in each row, differ significantly (P < 0,05)
** Means with different exponent (1 2 3 4) in each column differ significantly (P < 0,05)

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Inheritance and Stability of some Agronomic Traits of African Yam Bean (*Sphenostylis stenocarpa* (Hochst ex. A. Rich) Harms)

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ABSTRACT

African yam bean (AYB), *Sphenostylis stenocarpa*, an indigenous African pulse has immense nutritional significance. The understanding of inheritance pattern and stability status of agronomic trait is primary to their genetic improvement. Thirty AYB genotypes were evaluated for 100 seed weight (100SW), seed weight per pod (SWP), days to 50% flowering (D50F) and days to seedling emergence (DSE) in a randomized complete block design of three replications. This study was conducted at Ibadan, Ikenne, Mokwa and Ubiaja in Nigeria. Genotypic variation was significant ($p \leq 0.05$) for all characters except DSE. Location and Genotype x Location interactions were significant ($p \leq 0.05$) for the four characters. D50F had the least genotypic and phenotypic coefficient of variation (GCV and PCV) of 10.26% and 11.05% , respectively. The highest GCV, PCV and genetic advance occurred in SWP at 34.55%, 37.88% and 64.94% , respectively. Broad sense heritability ranged between 77.61% (DSE) to 89.07% (100SW). Repeatability was highest (13.83%) in 100SW. The performance of TSs87, TSs91 and TSs125 was highly significant for DSE and 100SW. The joint regression analysis identified TSs24 and TSs82 as the most stable genotypes for DSE and SWP with regression coefficient (b_i) of 0.94 and 0.97 and deviation from linearity (S_{di}^2) of 0.028 and -0.028 respectively. The most stable genotypes for D50F and 100SW were TSs61 and TSs84 with b = 1.015 and 1.017 respectively. The study revealed potential breeding values of four agronomic traits in AYB.

Keywords: African yam bean, accessions, underutilized species, agronomic traits, joint regression analysis.

Introduction

Global survey of useful crops identified 7,000 plant species; however, only about 150 are traded on the significant global scale (Padulosi *et al.* 2006). For example, over 50 percent of the global requirement for proteins and calories are met by just three: maize, wheat and rice. The policy which limits research and utilization on the few crops has greatly promoted the neglect of other crops. Benefits from the neglected and underutilized species includes, enhancement and support of food security, income generation, environmental health, food culture etc. Among the notable attending problems to their neglect are unquantifiable loss of their genetic resources, valuable cultures, etc. through genetic erosion, disappearance from cultural meals and under or none utilization of their potentials. The later would have not been a problem if awareness of these species is improved.

Sphenostylis stenocarpa (African yam bean) is one of such species; the record of the extent of its loss and rescue of its genetic resources in Africa is unknown (Adewale *et al.*, 2012). The seeds and tubers are the two organs of economic importance providing food for human and livestock. For the humans in Africa, there is cultural and regional preference for each of the two economic products (Potter, 1992; Nwokolo, 1996).

Yield had been on focus in most stability assessment; however, other quantitative traits are likewise influenced by the environment (Aremu *et al.*, 2007; Adewale *et al.*, 2010; Sameh *et al.*, 2011). Crop yield improvements have been achieved through directional selections for yield components (Akbar

and Kamran, 2006). Therefore, an assessment of some genetic parameters and stability of some quantitative traits whose contribution to grain yield had been remarked positive (Adewale, 2011) is primary for African yam bean (AYB) improvement.

The inconsistency in the performance of the same genotype in many environments for specific trait makes prediction of its phenotypic performance across a wide environment impossible (Perkins and Jinks, 1968). The same has grossly affected crop breeding programme formulation (Kang *et al*., 1987), such that phenotypic performance assessment of genotypes for various targeted environments has become a necessary component any breeding programme. If there were no genotype by environment (G x E) interaction associated with the genotype-environment system relevant to a breeding objective, selection would be greatly simplified because the 'best' genotype in one environment would also be the 'best' genotype for all target environments. If such scenario had been real, crop varieties trial would be conducted in a single replication at only one location to provide universal results (Gauch and Zobel, 1996).

The impossibility of such phenomenon therefore underscores the importance of G x E in varieties evaluations. Reliable recommendation and release of genotypes for specific environment with higher confidence can only be achieved through the understanding of the adaptability or stability of each genotype to respective environment. One of the most popular univariate and extensively used method (Ariyo, 1990; Makinde and Ariyo 2011; Sameh *et al*., 2011; Yonas, 2014) for determining stability across environments has been the joint regression analysis approach. It was proposed by Yates and Cochran (1938) and further developed by Finlay and Wilkinson (1963) and Eberhart and Russell (1966). The popularity among other methods may be due to its simplicity of calculation and application (Becker and Leon, 1988), moreover, it provides a conceptual model for genotypic stability.

The proportion of the phenotypic expression of a genotype that is genetic is key to the determinant of how much of the variation in the trait is linked to genetic factors and how much of the genetic advance is passed to the offspring. Simply, if heritability is greater than zero, then the inherent genetic component of the genotype has a measure of contribution to the phenotype in the respective environment. For every quantitative trait, the genetic component is a function of the heritability, but the phenotypic performance of individual genotype is dependent on the measure of flexibility or elasticity with respect to specific environment. Therefore, the breeding value of a quantitative trait is partly dependent on heritability and stability of the genotype.

Study on inheritance and stability of agronomic traits of African yam bean has not been attempted. The present study identified four quantitative traits: days to seedling emergence, days to 50% flowering, 100 seed weight and seed weight per pod to be under G x E interaction. Understanding their heritability and stability would be good information for subsequent breeding programme plans, especially for grain yield. Initial test for relationship of these traits with grain yield (Adewale, 2011) revealed high and positive correlation.

Materials and Methods

Thirty accessions of AYB were selected across some generated clusters obtained from an initial characterization of eighty AYB accessions. The 30 AYB accessions were presented for a multi-locational evaluation to understand the stability and heritability of four agronomic traits. This experiment was laid out in Randomized Complete block Design of three replications in four agro-ecologies within Nigeria: namely Ibadan, Ikenne, Mokwa and Ubiaja. The ecological properties of the four locations are presented in Table 1. Three seeds were planted per hill at 1metre apart. Thinning was done two weeks after planting to reduce plants/hill to two and the seedlings were staked three weeks after planting. Nuvacron (2.5ml L^{-1}) was applied at interval of two weeks from flowering inception to control the floral and pod pests. Manual weeding was done regularly to keep the field free of weeds. Data was collected on days to seedling emergence, days to 50% flowering, 100 seed weight and seed weight per pod.

Data were subjected to Analysis of Variance (ANOVA) using PROC GLM in SAS for each of the locations and then for the combined locations. The means of genotypes were compared using critical difference (CD), using the formula of Singh and Chaudhary (1985):

$$CD = (2\ MS_e/r)^{1/2} \times t$$

where; MS_e is the error mean square, r is the number of replicates and t is the tabulated value at 5% or 1% level of significance for the degree of freedom of error mean square.

The phenotypic variance (σ^2_P) was estimated following the method of Toker (2004) as:

$$\sigma^2_P = \sigma^2_G + \sigma^2_{GL}/L + \sigma^2e/rL$$

where;

G, L and r are genotypes, locations and replication respectively. σ^2_G, σ^2e and σ^2_{GL} are components of variance for genotype, error and the interaction between genotypes and location respectively.

Broad sense heritability (Hb) was estimated following Tenkouano *et al*. (2002) and Toker (2004) as:

$$Hb = \sigma^2_G / (\sigma^2_G + \sigma^2_{GL}/L + \sigma^2e/rL)$$

Phenotypic and genotypic coefficients of variation were estimated by the formula suggested by Gopal (2001) as:

Phenotypic Coefficient of Variation (PCV) = $100\sqrt{\sigma_p^2}/x$

Genotypic Coefficient of Variation (GCV) = $100\sqrt{\sigma_g^2}/x$

where; x is the population mean, σ^2_p is the addition of the genotypic mean square and the error mean square and σ^2_g is the genotypic mean square – error mean square/number of replications.

Repeatability (r_c) was estimated according to the formula presented by Ortiz and Ng (2000), as follows:

$r_c = \sigma^2_G/(\sigma^2_L + \sigma^2_{GL})$

where; σ^2_G is the variance of the genotypes, σ^2_L is the variance of the environment (Location)

σ^2_{GL} is the variance of the genotype (Genotype) and the environment (Location).

Joint Regression Analysis (See equation below) was performed following the approach of Eberhart and Russell (1966)

$Y_{ij} = m_i + \beta_i I_j + \delta_{ij}$

Where, Yij = Mean of the ith genotype at the jth environment (i = 1, 2, 3, 4, 5……30, j = 1, 2, 3, 4)

m_i = The mean of ith genotype over all the environments

βi = The regression coefficient that measures the response of ith genotype to varying environment

δ_{ij} = The deviation from regression of the ith genotype of jth environment

Ij = The environmental index obtained by subtracting the regression of the ith genotype the grand mean from the mean of all genotype at jth environment.

Based on the recommendation of Eberhart and Russell (1966), the significant Genotype x Location interaction component from the initial ANOVA was further partitioned into linear/predictable (Locations and Genotypes x Locations) and non-linear/unpredictable (Pooled Deviations) components. Mean squares for both components and each of the accessions were tested against the pooled error mean square of the four traits. Moreover, all sources of variation in the linear component were further ascertained for significance by testing with the pooled deviation, following the Gonçalves *et al.* (2003), Akcura *et al.* (2006) and Islam *et al.* (2006). Following the procedure of Kenga *et al.* (2003), standard errors of regression (b_i) values were generated to test the significant deviation of b from 1.0. Moreover, the test for significant deviation from regression (S^2d_i) from zero was done using F-test involving the comparison of the mean squares due to deviations from regression with pooled error mean squares for each of the four traits.

Results and Discussion

In Table 2, the 30 accessions differed significantly (P≤0.05) from each other for most of the traits in each of the locations. Under the combined analysis of variance, days to seedling emergence (DSE) did not differ among the thirty genotypes. However, highly significant (P≤0.001) variation existed among the 30 accessions for the other traits (Table 2). The four locations differed significantly (P≤0.001) and Genotype by Location (G x L) interaction equally differed significantly (P≤0.05) for the four traits. The proportions of the G x L for each of the traits were less than 10%; the least (1.78%) and the highest (5.17%) occurred in days to 50% flowering (D50F) and one hundred seed weight (100SW), respectively. The very low G x L and very high heritability observed for D50F in this study re-emphasize earlier remarks (Upadhyaya *et al.*, 2002; Aazami and Jalili, 2011, Adewale *et al.*, 2012) that flowering trait are less influenced by the environmental factor. This may have informed the common prominence of flowering traits as effective discriminatory descriptor component for intra-specific morpho-genetic characterization.

Mean number of days to seedling emergence and attainment of 50% flowering among the 30 AYB accessions were approximately 6 and 95, respectively. Moreover, mean weight of seeds per pod (SWP) and that of 100SW were 3.35g and 23.62g, respectively (Table 3). Broad sense heritability of the four traits was high, ranging from 77.61% (DSE) and 89.07% (100SW); the highest repeatability (13.83%) in the study occurred in 100 seed weight. Generally from Table 3, the phenotypic coefficients of variation (PCV) were higher than the genotypic coefficients of variation (GCV). Proportionately, the contributions of the genetic component to the phenotypic variation in the four traits were high, ranging between 86.38 and 94.38. Relevance of the ratio of GCV to PCV as a reliable guide to selection of genotypes has been largely reported (Ortiz and Ng, 2000; Kaushik *et al.*, 2007; Adewale *et al.*, 2010). Repeatability is a useful tool for quantifying the extent to which individual performances remain consistent over time and space (Arnold, 1994). Therefore, a reliable breeding selection programme for AYB can be based on the following criteria: high GCV: PCV ratio, high broad sense heritability, high genetic advance and high repeatability.

Partitioning of the G x L interaction was done by Eberhart and Russell (1966) regeneration method. The identified significant difference among the thirty accessions for the four traits in Table 4 simply indicates that the genotypes differed in their performances for the four traits. It is therefore possible to improve them through selection breeding programme (Yonas, 2014). The mean square due to environment (linear) was significant, indicating that differences existed between environments. However, the significant interaction effect of the accessions across the four locations (observed from Table 4) would complicate any selection programme based on the differential performances of the accessions because accessions at one environment did not have correlated at the other environments.

The G x L interaction component was further partitioned into linear (Location and Genotype x

Locations = predictable) and non-linear (pooled deviations = unpredictable) components. Mean squares for both components were tested by the pooled error mean square. The linear component was highly significant, indicating that the predictable components had a vast share in the G x L interactions. The high contribution of the linear component to G x L interaction is of great practical importance, implying that there are differences among linear regression coefficients for each accession with respect to each of the four traits.

The G × L (linear) was found to be non significant (Table 4) when tested against pooled deviation. This ought to indicate the preponderance of non-linear component; however, the test for significance of the non-linear (pooled deviation) component using the pooled error was also not significant for the four traits studied. According to Islam *et al.*(2006) and Akcura *et al.*(2006), non significance of the G x L (linear) component seem to reveal that the source of the G x L interaction is not well defined, hence, prediction for responses of the genotypes to the environments for DSE, D50F and 100SW would be difficult. However, mean square due to G × L (linear) and Location (linear) for SWP were found to be significant ($P<0.01$) when tested against pooled deviation (Table 4). According to Da and Saleh (2003), the significance of the two sources of variation is an indication that there is heterogeneity in the regression coefficients of the genotypes. In Table 4, the accessions with significant ($P<0.01$) performances for DSE include TSs23, TSs81, TSs86, TSs87, TSs96, TSs118 and TSs125. Other significant ($P<0.01$) accessions are TSs91 (D50F) and TSs96 (100SW).

The stability analysis showed a wide variation among accessions; some exhibited wide adaptation while other showed specific adaptation either to favorable or un-favorable environments. Days to seedling emergence and 50% flowering would be meaningful and desirable when their mean values are low. Therefore from Table 5, days to reaching seedling emergence ranged between 5.67(TSs9) and 7.00(TSs61). Among the thirty accessions, the least number of days to attaining flowering was 90.75(TSs48) and the highest (103.83) was observed in TSs91. Based on the recommendation depicting stability by Eberhart and Russell (1966), genotype(s) with $b_i = 1.0$ and $S^2d_i = 0$ are approved to be stable. For DSE in Table 5, seven accessions had significant b_i values >1.0 (1.487 – 1.954), six other accessions had b_i values that were significantly <1.0 (0.016 – 0.548). For D50F, only three and one accessions respectively had b_i values >1.0 and <1.0 respectively. Coupled with lower mean value (desirable for DSE and D50F), some of the accessions with stable characteristics for DSE were: TSs 24 and TSs89. TSs48 and TSs61 was stable accessions for D50F. TSs86 and TSs96 (DSE) and TSs91 (D50F) had $b_i > 1.0$ and $S^2d_i > 0$ (Table 5); their adaptability with respect to earlier days of seedling emergence and 50% flowering was to favorable or high yielding environment. TSs9, TSs10, TSs33 and TSs67 had $b_i > 1.0$ and $S^2d_i < 0$ for DSE, this is an indication that their response to earlier germination is enhanced under a harsh or unfavourable environment.

Desirability of a hundred seed weight and seed weight per pod is in the higher mean value.TSs10, TSs67, TSs81, TSs91, TSs101 and TSs125 had greater than the mean value for 100SW, $b_i = 1.0$ and $S^2d_i = 0$ (Table 6). Other accessions with stable characteristic for 100SW but with lower than the grand mean of 23.62g were: TSs9, TSs23, TSs48 and TSs48. Moreover, TSs86, TSs91, TSs93, TSs94, TSs96, TSs104B and TSs125 were stable with respect to SWP; because they had higher mean value and their b_i and S^2d_i were significantly equals to 1.0 and 0.0 respectively. TSs89 produced the highest seed weight per pod, however, the b_i was significantly <1.0. Moreover, TSs96 whose 100 seed weight of 26.15g was much higher than the mean (23.62g) had significantly $b_i > 1.0$ and $S^2d_i > 0.0$(Table 6). For these traits, TSs89 and TSs96 were unstable and their performances over the four environments cannot be predicted. Moreover, the adaptive response of TSs58 and TSs86 (100SW) and TSs10 (SWP) for better performances would be enhanced in favourable environment. TSs33 was also identified to be favoured for higher seed weight per pod in poorly enhanced environment.

As remarked by Makinde and Ariyo (2011) and Yonas (2014), stable genotypes with desirable characteristics (such as earliness in DSE and D50F and high yield in 100SW and SWP) could be selected as parent for further improvement of the trait of concern. Although the 30 AYB accessions differed in stability for the four studied characters across the different environments, the potential performances and stability for the four traits were not mutually exclusive. The significantly higher than zero S^2d_i value obtained for TSs125 (DSE), TSs91 (D50F) and TSs96 (100SW), according to Kenga *et al.* (2003) suggests that their response were not adequately described by the linear regression and that most of the rest accessions exhibited general adaptability in the environments. The heterogeneity in response of the accessions to the environments earlier remarked was further confirmed by the differential and significant b_i values (< 1.0, 1.0 and >1.0) observed for the accessions for the four traits.

The concept of repeatability is expressed as the correlation between measures of a given trait in an individual genotype repeated in time or space (Benin *et al.,* 2005). This coefficient expresses the proportion of total variation that is explained by the variation of the genotype and those attributable to the environment (i.e. the environment plus G x E). High values of this coefficient according to Falconer and Mackay (1996) indicate that the genotype or the trait is expressed with high stability. In this study therefore, the sequence of stability of the four traits by comparison is: 100SW>SWP>DSE>D50F.

Table1. Description of the test location in terms of the coordinates, agro-ecology and total monthly rainfall in 2007 cropping season.

Locations	**Ibadan**	**Ikenne**	**Mokwa**	**Ubiaja**
Coordinates	**7.5^{0}N, 3.9^{0}E**	**6.9^{0}N, 3.7^{0}E**	**9.3^{0}N, 5.05^{0}E**	**6.65^{0}N, 6.38^{0}E**
Agro-ecology	**Forest-savanna transition zone**	**Lowland Humid forest**	**Southern Guinea Savanna**	**Humid Rainforest**
Months	**Total monthly rainfall (mm)**			
January	-	-	-	-
February	0.05	-	-	42.0
March	15.9	38.4	10.0	71.8
April	70.7	16.0	116.0	111.3
May	201.27	141.0	202.5	215.2
June	308.25	409.7	127.5	205.4
July	145.5	286.8	106.0	298.2
August	121.55	144.4	414.0	150.1
September	264.75	313.6	363.0	416.8
October	203.95	170.2	39.5	185.2
November	9.85	70.1	-	0.7
December	0.05	4.5	-	21.5
Total	1341.82	1594.7	1378.5	1718.2
Mean	111.82	132.89	114.88	143.18

* Source: Geo-Spatial Laboratory at IITA, Ibadan,

Table 2. Summary of the analysis of variance of four agronomic traits of African yam bean

Sources of variation		Mean Square			
	Df	DSE	D50F	100SW	SWP
Ibadan					
Genotypes	29	1.32ns	76.04***	22.81***	1.13**
Error	58	1.68	13.84	5.07	0.44
Ikenne					
Genotypes	29	1.002***	39.48ns	17.06***	1.06*
Error	58	0.24	51.25	6.16	0.63
Mokwa					
Genotypes	29	1.06ns	95.63ns	42.87**	1.09*
Error	58	1.3	63.61	15.73	0.62
Ubiaja					
Genotypes	29	1.6***	32.75***	18.35**	0.78*
Error	58	0.4	6.45	7.6	0.45
Combined Location					
Locations	3	27.36***	2626.08***	302.16***	19.69***
Genotypes	29	1.32ns	95.77***	44.39***	1.34***
Genotypes x Location	87	1.22*	49.38*	18.9***	0.91**
Error	232	0.91	33.79	8.64	0.54
GEI Proportion (%)		4.08	1.78	5.17	4.15

DSE – Days to seedling emergence, D50F – Days to 50% flowering, 100SW – 100-seed weight, SWP – Seed weight per pod
GEI Proportion (%) – Proportion of the total variance due to Genotypes x Location interaction
*, **, *** - Significance at P ≤ 0.05, 0.01 and 0.001

Table 3. Estimates of some genetic parameters of four agronomic traits of 30 African yam bean genotypes

Agronomic traits	Mean	GCV	PCV	GCV:PCV	Hbs	r_c
Days to seedling emergence (days)	6.17	18.26	21.14	86.38	77.61	4.62
Days to 50% flowering (days)	95.35	10.26	11.05	92.85	86.33	3.58
100-seed weight (g)	23.62	28.20	29.88	94.38	89.07	13.83
Seed weight per pod (g)	3.35	34.55	37.88	91.21	83.10	6.25

NB: Hbs – Broad sense heritability (%), GCV – Genotypic coefficient of variation (%),
PCV – Phenotypic coefficient of variation (%), r_c – Repeatability (%)

Table 4. Actual sources of the variation due to G x L interaction derived through partitioning for the estimation of stability parameters of the four traits

Source of Variation	DF	DSE	D50F	100SW	SWP
Genotypes	29	1.76**	62.01**	29.30**	1.12**
GxL	87	1.00**	46.32**	19.55***	0.71**
Location + (GxL)	90	1.53**	64.76**	28.33**	1.05**
Location(Linear)	1	27.36***	3161.84***	270.44***	20.56***
Genotype x Location (Linear)	29	0.22	10.64	8.24	0.43**
Pooled Deviation	60	0.49	16.06	7.33	0.11
TSs9	2	0.17	5.19	4.62	0.16
TSs10	2	0.03	6.75	2.76	0.63
TSs23	2	1.21**	0.49	3.25	0.04
TSs24	2	0.61	16.37	1.32	0.40
TSs33	2	0.38	23.60	15.60	0.13
TSs48	2	0.95	11.33	8.05	0.21
TSs49	2	0.38	20.60	14.14	0.16
TSs57	2	0.04	2.25	2.06	0.19
TSs58	2	0.29	10.47	6.20	0.12
TSs61	2	0.71	3.79	1.21	0.13
TSs67	2	0.19	12.31	5.17	0.03
TSs69	2	0.02	3.00	2.44	0.24
TSs81	2	1.21**	26.57	2.62	0.06
TSs82	2	0.57	24.01	7.53	0.18
TSs84	2	0.37	26.34	5.71	0.35
TSs86	2	1.49**	20.00	2.82	0.55
TSs87	2	4.29**	1.40	13.69	0.27
TSs89	2	0.61	1.82	16.63	0.34
TSs91	2	0.95	104.66**	10.52	0.50
TSs93	2	0.43	3.05	7.41	0.31
TSs94	2	0.33	5.03	4.37	0.19
TSs95	2	0.89	38.05	3.83	0.33
TSs96	2	1.88**	4.05	37.01**	0.02
TSs101	2	0.97	26.52	2.90	0.25
TSs104B	2	0.90	7.59	3.15	0.05
TSs109	2	0.13	17.27	2.97	0.10
TSs111	2	0.67	1.48	4.42	0.05
TSs116	2	0.28	56.53	7.36	0.22
TSs118	2	3.64**	1.31	2.37	0.12
TSs125	2	4.59**	0.01	17.79	0.50
Pooled Error	240	0.65	29.82	8.42	0.47

NB: Hbs – Broad sense heritability (%), GCV – Genotypic coefficient of variation (%),
PCV – Phenotypic coefficient of variation (%), r_c – Repeatability (%)

Table 5. Mean and Parametric stability estimates of Joint Regression Analysis for Days to Seedling Emergence and Days to 50% flowering of African yam bean

Genotypes	DSE				D50F			
	Mean (days)	b	SE(bi)	S^2d_i	Mean (days)	b	SE(bi)	S^2d_i
TSs9	5.67	0.739	0.092	-0.195	91.83	1.085	0.041	-7.849
TSs10	5.83	1.247	0.015	-0.266	94.83	1.090	0.046	-6.284
TSs23	6.42	1.633	0.662	0.325	93.00	0.791	0.012	-12.547
TSs24	5.75	0.938	0.337	0.028	96.00	1.648	0.072	3.339
TSs33	6.08	1.954	0.209	-0.089	93.50	1.017	0.086	10.561
TSs48	6.00	0.816	0.520	0.195	90.75	1.048	0.060	-1.703
TSs49	6.33	1.024	0.207	-0.090	94.33	0.985	0.081	7.560
TSs57	5.50	0.755	0.019	-0.262	93.75	0.628	0.027	-10.786
TSs58	6.25	1.487	0.159	-0.134	93.83	1.207	0.058	-2.562
TSs61	7.00	1.548	0.387	0.073	91.67	1.015	0.035	-9.247
TSs67	6.08	1.259	0.106	-0.183	97.25	1.225	0.062	-0.725
TSs69	6.25	1.239	0.009	-0.271	97.33	0.740	0.031	-10.035
TSs81	6.42	1.300	0.663	0.325	96.42	1.196	0.092	13.532
TSs82	6.33	0.321	0.314	0.007	95.50	0.702	0.087	10.971
TSs84	5.83	0.967	0.203	-0.094	96.00	0.919	0.091	13.301
TSs86	6.08	1.535	0.817	0.465	97.25	1.318	0.080	6.962
TSs87	6.83	1.125	2.352	1.866	101.83	1.012	0.021	-11.633
TSs89	6.08	0.938	0.337	0.028	95.83	0.753	0.024	-11.212
TSs91	6.33	0.418	0.522	0.196	103.83	1.804	0.182	91.624*
TSs93	6.50	0.792	0.235	-0.065	93.58	1.172	0.031	-9.983
TSs94	5.83	0.016	0.183	-0.113	99.25	0.718	0.040	-8.011
TSs95	6.08	0.309	0.485	0.163	94.42	1.423	0.110	25.009
TSs96	6.00	1.604	1.028	0.658	92.92	0.942	0.036	-8.983
TSs101	6.33	1.271	0.532	0.206	93.83	0.902	0.092	13.488
TSs104B	5.92	0.751	0.494	0.172	93.25	1.025	0.049	-5.445
TSs109	6.17	0.471	0.072	-0.214	96.50	0.980	0.074	4.238
TSs111	6.25	1.803	0.369	0.057	96.17	1.008	0.022	-11.561
TSs116	5.83	0.548	0.154	-0.139	94.00	0.749	0.134	43.495
TSs118	6.50	0.520	1.997	1.542	98.58	0.108	0.020	-11.722
TSs125	6.50	0.670	2.517	2.016*	93.50	0.791	0.002	-13.023
Grand Mean	6.17				95.36			
CD (5%)	1.52				9.30			

*, **, *** - Significance at P ≤ 0.05, 0.01 and 0.001

Table 6. Mean and parametric stability estimates of Joint Regression Analysis for 100 Seed weight and Seed weight per pod of African yam bean

Genotypes	100SW				SWP			
	Mean (g.)	b	SE(bi)	S^2d_i	Mean (g.)	b	SE(bi)	S^2d_i
TSs9	19.64	1.123	0.131	0.934	3.25	0.269	0.487	-0.042
TSs10	24.00	0.705	0.101	-0.922	3.56	1.909	0.956	0.420
TSs23	23.48	0.634	0.110	-0.431	3.01	1.364	0.249	-0.163
TSs24	23.99	1.917	0.070	-2.359	3.38	2.177	0.761	0.192
TSs33	23.35	2.254	0.240	11.921	3.97	3.499	0.441	-0.072
TSs48	22.94	0.966	0.173	4.372	2.88	1.480	0.554	0.005
TSs49	18.51	1.281	0.229	10.454	3.23	1.109	0.478	-0.049
TSs57	23.64	1.495	0.087	-1.623	3.28	1.019	0.528	-0.014
TSs58	24.19	2.417	0.151	2.521	3.12	1.703	0.425	-0.081
TSs61	22.28	0.281	0.067	-2.476	3.17	0.762	0.440	-0.073
TSs67	26.83	0.776	0.138	1.486	3.18	0.245	0.217	-0.173
TSs69	23.60	1.513	0.095	-1.242	2.89	1.032	0.595	0.037
TSs81	24.40	1.205	0.098	-1.062	3.56	1.436	0.304	-0.142
TSs82	23.76	2.092	0.167	3.844	3.43	0.970	0.508	-0.028
TSs84	23.33	1.017	0.145	2.025	3.33	1.124	0.712	0.142
TSs86	25.18	2.077	0.102	-0.864	3.65	1.212	0.900	0.350
TSs87	23.19	1.851	0.225	10.005	2.94	1.503	0.623	0.061
TSs89	25.57	-1.066	0.248	12.952	4.22	-0.296	0.703	0.134
TSs91	23.95	1.173	0.197	6.836	3.47	1.009	0.856	0.297
TSs93	23.80	1.609	0.166	3.728	3.53	0.769	0.672	0.105
TSs94	21.78	-0.495	0.127	0.689	3.54	-0.895	0.524	-0.017
TSs95	25.98	0.335	0.119	0.147	3.57	0.001	0.697	0.128
TSs96	26.15	-1.759	0.370	33.323*	3.82	1.014	0.163	-0.187
TSs101	23.81	0.732	0.104	-0.785	3.15	0.662	0.606	0.046
TSs104B	22.96	0.309	0.108	-0.534	3.48	0.830	0.261	-0.159
TSs109	23.03	1.417	0.105	-0.711	2.96	1.181	0.373	-0.110
TSs111	21.64	1.550	0.128	0.741	2.75	1.117	0.274	-0.154
TSs116	22.43	1.812	0.165	3.675	3.28	0.490	0.563	0.012
TSs118	23.02	0.074	0.094	-1.311	3.17	0.406	0.425	-0.081
TSs125	28.29	0.705	0.256	14.109	3.69	0.901	0.854	0.295
Grand Mean	23.62	-	-	-	3.35	-	-	-
CD (5%)	4.70	-	-	-	1.17	-	-	-

*, **, *** - Significance at P ≤ 0.05, 0.01 and 0.001

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A Search for Candidate Gene for Cowpea Powdery Mildew Resistance in the Southern Guinea Ecology of Nigeria

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ABSTRACT

The need to identify candidate gene for resistance to powdery mildew (PM), a major fungal foliar disease of cowpea in Southern guinea savannah of Nigeria has necessitated this research. An experiment involving 13 cowpea genotypes was laid out in a randomized complete block design (RCBD) of two replications at the Botanical Garden, University of Nigeria, Nsukka, Nigeria. Each genotype was scored for susceptibility to the disease. Four parents were afterward selected for progeny generation through a 2 x 2 factorial mating design. The F_1 hybrids were evaluated in RCBD of two replications on the field for powdery mildew resistance. The scored data was transformed by arcsine method before subjection to analysis in SAS (version, 9.3). Analysis of variance (ANOVA) revealed significant ($P<0.01$) differences among the 13 genotypes in their susceptibility to PM. The male, female and the interaction of both significantly ($P<0.001$) differed. Additive genetic variance (510.07) was higher than the dominance genetic variance (387.67). Additive gene action was prominent in this study. The broad and narrow sense heritability estimates were: 99.9% and 56.8% respectively. The average degree of dominance was 1.23 and the genetic advance was 62.09. Heterosis which signifies resistance to PM was observed in the crosses between Nsukka-BA x IT89KD-374-57, IT90K-59 x IT89KD-374-57 and IT90K-59 x Nsukka-1B. The identified resistant genotypes (IT90K-59, Nsukka-BA and IT89KD-374-57) would be resources for further breeding programme. Powdery mildew cowpea resistant cultivar development could be achieved through hybridization programme since the major contribution to the inheritance of the trait was additive.

Keywords: Powdery mildew, inheritance, genetic advance, gene action, F1 hybrids, heterosis.

Introduction

Powdery mildew (PM) is an important fungal disease in several legumes. It is caused by *Erysiphe polygoni* (Braun, 1987). It is an obligate pathogen that establishes lasting interactions with their host tissues. There are about 700 PM species capable of colonizing approximately 10,000 plant species (Braun and Cook 2012). In cowpea, *Podosphaera phaseoli* (syn. *Sphaerotheca phaseoli*) has been indicted as the causal organism (Soylu *et al.*, 2004).

Powdery mildew, a biotrophic fungus has a wide distribution. It is particularly important in climates with warm, dry days and cool nights (Smith *et al.*, 1996; Sillero *et al* 2006). Gritton and Ebert (1975) summarized the economic importance of powdery mildew as causing yield and quality losses. Severe infection may cause 25-50% yield losses (Munjal *et al.*, 1963; Warkentin *et al.*, 1996). Reddy *et al.*, (1994) and Shambarkar *et al.* (1997) reported up to 40% loss in Mung bean (*Vigna radiata*) and 45% in sesame (*Sesamum indicum*) respectively. Emechebe and Florini (1997) reported that the damages due to powdery mildew on cowpea in the Sudan savanna (a drier agro-ecology) of Nigeria were moderate. Efficiency of powdery mildew is highly dependent on the weather (Wongpiyasatid *et al.*, 1999).

Control of powdery mildew has been by foliar application of chemicals (Ransom *et al.* 1991; Lewellen

and Schrandt 2001; Utkhede *et al*. 2001). Environmental risks and additional production cost of chemical purchase is associated with chemical control method. Hence, the most effective measure to control such a disease would be to breed for resistant varieties.

The genetics of resistance to powdery mildew has been studied in some legumes. Lohnes and Bernard (1992) identified *Rmd* locus to confer resistance to PM in Soybean (*Glycine max*). However, in peas (*Pisum sativum* L.), resistance to PM was reported to be controlled by several separate recessive genes (Heringa *et al*. 1969; Timmerman *et al*. 1994). The report of Tiwari *et al*. (1997) indicted polygenic inheritance for the resistance of the disease in pea but resistance to the same disease in common bean (*Phaseolus vulgaris*) was controlled by two major dominant genes which interact via double recessive epistasis (Rezende *et al*.,1999).

Report of the incidence of Powdery mildew in Nigeria is quite scanty; however, its economic importance could be devastating where the climatic condition favours the development of the organism. The Southern guinea savanna ecology is an important cowpea producing zone in Nigeria (Abayomi *et al*., 2008).

A worthwhile improvement programme for cowpea powdery mildew resistance would need information on the genetic diversity of various genotypes and the gene action controlling the trait. The present study therefore seek to understand the differential susceptibility of different cowpea genotypes to powdery mildew infection, identify candidate genes for resistance in parents and understand the gene action responsible for resistance or susceptibility in the studied genotypes.

Materials and Methods

Susceptibility and/or resistance of thirteen cowpea varieties (Table 1) to powdery mildew were assessed in the field at the Botanical garden of the University of Nigeria, Nsukka. Their seeds were planted in prepared plots at a spacing of 0. 5 x 0. 5m. The experiment was laid out in a Randomized Complete Block Design (RCBD) with three replications. Basic cultural practices were carried out to maintain the plots and the plants.

Symptom of powdery mildew was observed and the extent of attack was estimated following the method of James (1971). Quantity of mycelia growth under and on the surface of the leaves was scored per plant in each plot. Scores were rated as percentage of infection per plant as: 0 (no visible infection), 1 (up to approximately 25% leaf coverage with mycelia), 2 (approximately 26– 50% leaf coverage), 3 (approximately 51–75% leaf coverage), 4 (approximately 76–100% leaf coverage). An average score from the five sampling unit in each plot was taken.

An infection index was calculated following the equation of Wheeler (1969) as:

$$\text{Infection index} = \frac{\text{number of numeric rating}}{\text{total number of plant}} \times \frac{100}{\text{maximum disease category}}$$

Based on the varied performance of the different genotypes in the initial screening, four genotypes: two elites (IT90K-59 and IT89KD-374-57) and two local (Nsukka-BA and Nsukka-1) varieties were selected for cross breeding and progeny generation. A 2 x 2 factorial mating design was employed for the crossing programme and the expected mean square of each component in the 2 x 2 fixed model factorial mating design was presented in Table 2. The generated F_1 hybrids were evaluated on the field in RCBD of two replications. Resistance to powdery mildew was conducted following the earlier screening protocol.

Percentage infection ranged between zero (0%) to 90%. The data was transformed using Arcsine technique to normalize and stabilize the variances as recommended by Gomez and Gomez (1984) and to fit it to parametric format (Fowler *et al.*, 2008). Analysis of variance was conducted using SAS version 9.3 (SAS Institute, 2012) to partition the sources of variations in the two experiments.

Differences among treatments in the two experiments were assessed with the Duncan Multiple Range test at the 0.05 significance level. Analysis of variance of the second experiment was done following the statistical linear model of Comstock and Robinson (1948, 1952):

$$Yijk = \mu + mi\,fj + mf\,(ij) + Rk + eijk$$

Where **μ** is the grand mean, **m** is male, **f** is female and R is replication. Moreover, *i, j* and *k* are the numbers of male, female and the replications respectively.

The components of phenotypic variation includes: Additive variance ($\sigma^2 A$), Dominance variance ($\sigma^2 D$) and environmental variance ($\sigma^2 E$) Variances of the male, female and their interaction were estimated from the equation below:

$$\sigma^2 m = \frac{MSm - MSe}{fr} = \sigma^2 \text{G.C.A} = \frac{1}{2}\sigma^2 \text{A}$$

$$\sigma^2 f = \frac{MSf - MSe}{mr} = \sigma^2 \text{G.C.A} = \frac{1}{2}\sigma^2 \text{A}$$

$$\sigma^2 m \text{x} f = \frac{MSmf - MSe}{r} = \sigma^2 \text{S.C.A} = \sigma^2 \text{D}$$

Where:

$\sigma^2 e = MSe$; $\sigma^2 m$ and $\sigma^2 f$ = Variance for the general combining ability (GCA) for males and females respectively and $\sigma^2 m' f$ = Variance for the specific combining ability (SCA) of the hybrids.

Additive ($\sigma^2 A$) and dominance ($\sigma^2 D$) variances were estimated as:

$$\sigma^2 A = \frac{2\,\sigma^2 m + 2\,\sigma^2 f}{2} \text{ and } \sigma^2 D = \sigma^2 m \text{ x } f$$

The total genetic variation was calculated as:

$$\sigma^2 G = \sigma^2 A + \sigma^2 D$$

And phenotypic variance:

$$\sigma^2 P = \sigma^2 G + \sigma^2 E$$

Broad ($h^2 b.s$) and narrow sense ($h^2 n.s$) *heritabilities* were estimated from the following equations:

$$h^2 b.s = \frac{\sigma^2 G}{\sigma^2 2}, \quad h^2 \text{n.s} = \frac{\sigma^2 A}{\sigma^2 p}$$

$$\text{Average degree of dominance } (\bar{a}) = \sqrt{\frac{2\,\sigma^2 D}{\sigma^2 A}}$$

$$\text{Expected genetic advance } EGA: = \frac{h2n.s.}{(\sigma P) \text{ x } (i)}$$

$$\text{Expected genetic advance as a percent of mean (\%)} = \frac{EGA}{x} \text{ x } 100$$

Where, i = Coefficient of selection which is 2.06 at 5% selection intensity. σP = Phenotypic standard deviation and $\bar{x}$ = Mean

Heterosis (H) = $\overline{F1}$ - ($\overline{Pi}$ +$\overline{Pj}$ /2)

Where: $\overline{F_1}$ = F_1 Mean, $\overline{Pi}$ = Mean of parent one and $\overline{Pj}$ = mean of parent two.

Results

The analysis of variance for the preliminary screening revealed significant ($p<0.01$) variation among the 13 cowpea genotypes for percentage infection to powdery mildew infection (Table 3).Significantly lower mean infection were recorded for IT81D-985 (14.67), Brown Akidi(14.95) and IT89KD-245(15.39). The mean infection for IT95K-56, IT90K-277-2, Nsukka-BA, IT89KD-374-57 and L.25 were moderate ranging from 16.41 to 18.97. However, from Table 3 significantly higher mean infections were recorded for IT90K-59, IT91K-118-20, Nsukka-1and Nsukka-1W genotypes.

Analysis of variance for the factorial mating design revealed highly significant ($p<0.001$) differences among the males, the females and their progeny (Table 4). From Table 4, the mean infection for the parents and the progenies was 31.48%. The coefficient of variation for the experiment was 2.83%.

From Table 5, IT89KD-374-57 (a male parent) had the lowest mean infection of 16.7. Much higher and significant mean infection (46.26 and 37.55) was recorded for Nsukka-1 and Nsukka-BA, respectively. Powdery mildew infection was medium (25.41) for IT90K-59 (Table 5). The performance of the F_1 hybrids with respect to percentage infection with powdery mildew ranged between high susceptibility to high resistance. For example, Nsukka-BA x IT89KD-374-57 and IT90K-59 x IT89KD-374-57, respectively displayed low percentage infection of 12.92 and 20.49 (Table 5). Among the progenies, the highest infection (62.18) was observed in the cross between Nsukka-BA and Nsukka-1.

Various genetic estimates were presented in Table 6. Additive genetic variance (510.06) was much higher than the dominance genetic variance (387.67) in the approximate ratio of 10:8 (Table 6). Broad sense heritability (99.91%) was almost twice higher than the narrow sense heritability (56.77%). From Table 6, the average degree of dominance was 1.23 while the expected genetic advance was 62.1%.

Heterosis estimates for the four hybrids ranged between -8.88 to 30.21. The four genotypes exhibited significant positive and negative outperformance above their parents. Hybrids with IT89KD-374-57 as pollen parent consistently had low heterosis, however, heterosis estimates of hybrids with Nsukka-1 as paternal parent was not consistent (Table 7).

Discussion

The study shows considerable genetic variability among cowpea genotypes for powdery mildew resistance. The differential response of cowpea genotypes and hybrids further suggested that this character was under genetic control and should therefore be liable to genetic manipulation for improvement. The differential response to the same biotic environment of the screened genotypes seems to agree with the observation of Sultan (2001) that genotypes respond differently to different environment. Disease severity scores of powdery mildew varied from 14.67 for IT81D-985 to 30.323 for Nsukka-1W. IT81D-985, Brown Akidi and IT89KD-245 recorded a significantly superior mean performance for powdery mildew resistance and therefore could be used as donor parents for introgression of the powdery mildew resistance gene through hybridization programme.

IT89KD-374-57 was a product of research at the International Institute of Tropical Agriculture;

Ibadan Nigeria. Adjadi (1996) had earlier identified the genotype among others in a field evaluation trial to have higher capacity for grain production. However in this study, its selection and usage in the 2 x 2 factorial mating design seem to reveal its resistance quality to powdery mildew as a paternal parent. F_1 hybrids (Nsukka-BA x IT89KD-374-57 and IT90K-59 x IT89KD-374-57) in which the genotype was involved as a donor parent were respectively foremost in powdery mildew resistance in this experiment. The Nsukka-BA x IT89KD-374-57 had negative heterosis; corresponding to low powdery mildew infection, this supports the dominant theory of heterosis by (Jones, 1917). However, among the hybrids Nsukka-BA x Nsukka-1 was most susceptibility. Gene assortment after hybridization of the two local varieties could not produce a worthwhile resistant progeny for the disease. Superiority in performance of hybrids may be aided by the presence of elite gene(s) in either or both of the parents.

The present study showed that powdery mildew resistance in the tested cowpea genotypes was largely governed by additive gene effect. Although additive gene effect was predominant, there was an evidence of non-additive component as well. Powdery mildew resistance in Mungbean is governed by more than one gene whose effect are both additive and dominant (Gawande and Patil, 2003). Since genetic improvement by selection relies mainly on additive gene components, significant advances in breeding for powdery mildew could be made. Our finding agrees with that of Waraluk *et al*. (2009) on common bean. In this experiment, the additive genetic variance of male was relatively lower than that of the female; understanding the underlying genetic factor would be necessary. To this end, a complete diallel crossing programme may be proposed to unravel the proportion of the genetic component due to maternal effect.

A narrow and broad sense heritability of 56% and 99% respectively was observed in this study. The closeness of the broad and narrow sense heritability estimates suggests that the environmental influence on this trait is low. The narrow sense heritability estimates in this study is very high according to the recommendation of Robinson *et al*. (1949). The genetic advance of 62% observed in this study was equally very high based on the recommendation of Johnson *et al.,* (1955). The high heritability and genetic advance indicates additive gene action in the control of the trait; thus forestalling that the gene(s) conferring resistance to powdery mildew in cowpea is highly heritable and would respond to selection techniques.

Table 1. Genetic materials for the experiment and the source of collection

S/N	Genotypes	Codes	Source
1	IT90K-59	V1	IITA, Ibadan
2	IT81D-985	V2	IITA, Ibadan
3	Brown Akidi	V3	Nsukka
4	IT88D- 867-11	V4	IITA, Ibadan
5	IT95K-56	V5	IITA, Ibadan
6	IT90K-277-2	V6	IITA, Ibadan
7	IT91K-118-20	V7	IITA, Ibadan
8	Nsukka-1W(Local cultivar (White)	V8	Nsukka
9	Nsukka-BA(Black Akidi)	V9	Nsukka
10	Nsukka-1(Local cultivar Brown)	V10	Nsukka
11	IT89KD-245	V11	IITA, Ibadan
12	IT89KD-374-57	V12	IITA, Ibadan
13	L.25	V13	IAR&T, Ibadan

Table 2. Analysis of variance of factorial mating design (Fixed model)

Sources of variation	DF	SS	*MS*	*EMS*
Replications	*r-1*	$\frac{\Sigma Y..k^2}{mf} - \frac{Y..^2}{mfr}$		
Males (M)	*m-1*	$\frac{\Sigma Yi..^2}{fr} - \frac{Y...^2}{mfr}$	*MSm*	$\sigma^2 e + rf\sigma^2 m$
Females (F)	*f-1*	$\frac{\Sigma Y.j.^2}{mr} - \frac{Y...^2}{mfr}$	*MSf*	$\sigma^2 e + rm\sigma^2 m$
M.X F.	*(m-1)(f-1)*	$\frac{\Sigma Yij.^2}{r} - \frac{\Sigma Yi...^2}{r} - \frac{\Sigma .j.^2}{mr} + \frac{Y...^2}{mfr}$	*MSmf*	$\sigma^2 e + r\sigma^2 mf$
Error	*(mf-1)(r-1)*	$\sum Yijk^2 - \frac{\Sigma Yij.^2}{r} - \frac{\Sigma Y..k^2}{mr} - \frac{Y...^2}{mfr}$	*MSe*	$\sigma^2 e$
Total	*Mfr-1*	$\sum Yijk^2 - \frac{Y...^2}{mfr}$		

Table 3. ANOVA Summary and means of the thirteen tested genotypes

Source	DF	Mean Square
Genotypes	12	59.5805744**
Error	24	20.085913
		Means
Nsukka-1W		30.326a
Nsukka-1		23.544ab
IT91K-118-20		23.486ab
IT90K-59		20.234bc
Nsukka-BA(Black Akidi)		18.972bc
IT89KD-374-57		18.972bc
IT88D-867-11		17.790bc
IT90K-277-2		16.808bc
IT95K-56		16.408bc
L.25		16.408bc
IT89KD-245		15.397c
Brown Akidi		14.952c
IT81D-985		14.670c
CV (%)		23.496
Mean		19.074

Table 4. Factorial ANOVA for resistance to Powdery mildew in F1 hybrid of Cowpea

Source	DF	Mean Square
Rep	1	0.7953
Female	1	1747.3655***
Male	1	294.4861***
Male*Female	1	776.1307***
Nsukka-1W		30.326a
Error		0.7953
CV (%)		2.8328
Mean		31.4815

Table 5. Mean performances of the parents and the Hybrids

Code	Pedigree	Means
M1	Nsukka-1	46.2606a
M2	IT89KD-374-57	16.7025b
F1	IT90K-59	25.4144b
F2	Nsukka-BA	37.5488a
F1M1	IT90K-59xNsukka-1	30.3437b
F1M2	IT90K-59x IT89KD-374-57	20.4850c
F2M1	Nsukka-BA x Nsukka-1	62.1775a
F2M2	Nsukka-BA x IT89KD-374-57	12.9200d

M1, M2 - Males; F1, F2 – Females; F1M1, F1M2, F2M1 and F2M2 – Crosses between the female and the male parents

Table 6. Estimates of Genetic parameters

S/N	Items	Estimates
1	Additive Variance	510.0652
2	Dominance Variance	387.6677
3	Error Variance	0.79538
4	Broad sense heritability	99.91148
5	Narrow sense heritability	56.76674
6	Average degree of dominance	1.232912
7	Expected genetic advance	62.09446

Table 7. Heterosis Estimates of the four hybrids

Codes	Hybrids	Heterosis
F1M1	IT90K-59 x Nsukka-1	-0.09042
F1M2	IT90K-59 x IT89KD-374-57	0.218125
F2M1	Nsukka-BA x Nsukka-1	30.20458
F2M2	Nsukka-BA x IT89KD-374-57	-8.88563

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Nutrient Use and Uptake Efficiency in Wheat and Triticale Genotypes under Low and Optimum Input Conditions

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ABSTRACT

N, P and Zn content, harvest index and grain yield were used to evaluate diverse 32 wheat and triticale genotypes categorized in four groups i.e. *Triticum aestivum*, *Triticum durum*, synthetic wheat and triticale genotypes. The genotypes differed significantly for all the characters indicating considerable variation for improvement of these traits. The varieties LoK1 and HD2687 were having highest grain yield under low and optimum input conditions respectively, while HD 2687 showed maximum percent of increase over low input conditions. PBW343 and P7307 were having highest harvest index under low and optimum input conditions respectively, whereas Syn5 was found to show highest percent of increase over low input conditions. As nutrient use and uptake parameters are concerned, for N content triticale genotypes TL2963 and TL2967 showed highest content under low and optimum input conditions, while percent increase over low input conditions was found to be highest in HD2687 (285.96 per cent). Genotypes TL2966 and Syn36 were having highest P content under low and optimum input conditions respectively among all the four groups, while TL2969 responded better over low input conditions. Among all the four groups triticale genotype TL2963 showed trend having high content of zinc under both low and optimum input conditions, whether for percent of increase over low input conditions P7531 responded better. Path coefficient analysis revealed that harvest index followed by biological yield had the direct effect under both conditions.

Keywords: Nutrient use efficiency, wheat and triticale, N, P, Zn content in wheat, harvest index.

Introduction

Wheat (*Triticum aestivum* L.) is one of the most important cereal crops in the world. The area, production and productivity of wheat in India is approximately 28.30 million ha, 84.70 million tones, approximately 29.90 qtl/ha, respectively during the year 2010-11. The corresponding figures in Haryana are 25.15 lakh ha, 116.30 lakh tones, 46.24 qtl/ha (Anonymous, 2010-11). The increase in the use of nitrogen (N) and phosphorous (P) fertilizers between 1960 and 2000 by intensive agricultural practices has led to degradation of air and ground water quality (Tilman *et al*., 2001). Even though N is among the most abundant elements on earth, it is the critical limiting element for growth of most plants due to its unavailability (Graham and Vance, 2000). According to an estimate, about 54% soils in Haryana are deficient in zinc. Soils with extractable Zn less than 0.6% mg/ kg soil will require the application of particular nutrient to sustain production. About 50% of the soils used for cereal production in the world contain low levels of the zinc available to plants which reduces not only grain yield, but also nutritional quality of grains. Triticale is one of the synthetic amphiploid of wheat and rye which came into commercial cultivation. Synthetic wheat are produced by artificially crossing tetraploid durum wheat (*Triticum turgidum*, 2n=4x=28, AABB), donor of the A and B genomes, with *Triticum tauschii* (2n=2x=14DD). P is second only to N as the most limiting nutrient for the plant growth (Biellski, 1973, Vance *et al*., 2000). Thus, efficiency of wheat cultivars in N use has become increasingly important, because of the cost of N fertilizer and of the potential

for nitrate pollution of underground water and the atmosphere. Research has shown that modern semi dwarf cultivars respond more to available nitrogen than the old, tall cultivars, which translates into higher returns to farmers (Ortiz - Monasterio *et al*., 1997). In addition, semi dwarf wheats do not necessarily require more nitrogen than older cultivars at lower levels of fertility (Ortiz - Monasterio *et al*., 1997). Graham *et al*., (2000) reported that deficiencies of zinc are well known in all cereals and cereal growing countries. From physiological evidence reported elsewhere, it would appear that a critical level for zinc is required in the soil before roots will either grow into it or function effectively (Graham *et al*., 2000). Zinc efficient genotypes absorb more zinc from deficient soils, produce more dry matter and more grain yield but do not necessarily have the highest zinc concentrations in tissue or grain. Although high grain zinc concentration also appears to be under genetic control, it is not tightly linked to agronomic zinc efficiency traits and may have to be selected for independently (Graham *et al*., 2000).

Material and methods

Experimental material: Experimental material comprised four groups of genotypes, namely, *T. aestivum*, *T. durum,* Triticale and synthetic wheat. Each group consisted of 8 genotypes, thus making a total of 32 genotypes. The detail of experimental material is given below.

Environments: The experiment was conducted in following environments

Low input: On the basis of soil test the doses of fertilizer were corrected up to 60 kg N, 30 kg P_2O_5/ ha. In addition to this two irrigations were applied. First irrigation was applied on CRI stage and second irrigation was applied on flowering stage.

Optimum input: On the basis of soil test the doses of fertilizer were corrected up to 150 kg N, 60 kg P_2O_5/ha. In addition, four irrigations were applied, first irrigation was applied on CRI stage, second irrigation was applied on tillering, third on flowering stage, and fourth on dough stage.

Layout: The design was laid out in split plot design. Plot size was of single row of 3 m length. Observations were taken as 5 plants / entry / replication.

Estimation of N content and P content - 0.5 gram of finely grounded samples were taken in digestion tube and 10 ml sulfuric acid + perchloric acid in the ratio of 4:1 poured in digestion tube and left over night. The material was heated from 90 minutes at 160ºC and 30 minutes at 220ºC. After cooling, every digestion tube was filled with 30 ml distilled water and after shaking volume was made 50 ml. Then this end product was filtered into plastic bottle of 100 ml and such digests were analyzed for N content and P content.

Estimation of Zn content - 0.5 gram of finely grounded samples were taken in digestion tube and 20 ml nitric acid + perchloric acid in the ratio of 4:1 poured in digestion tube and left over night. The material was heated from 90 minutes at 160ºC and 30 minutes at 220ºC. After cooling, every digestion tube was filled with 30 ml distilled water and after shaking volume was made 50 ml. Then this end product was filtered into plastic bottle of 100 ml and such digests were analyzed for Zn content.

N content (%) - N content was estimated in plant sample following standard procedure of A.O.A.C. (1970).

N uptake (mg/plant) - N uptake was calculated by multiplying the N content in shoot by dry weight.

P content in shoot (%) - P content was measured following standard procedure of A.O.A.C. (1953)

P uptake (mg) - P uptake was calculated by multiplying the P content in shoot by dry weight.

Zn content (ppm) in shoot - Zn content was determined by Atomic Absorption Spectrophotometer, GBC 902 plus.Micronutrients uptake was calculated by multiplying content with dry yield of straw.

The N,P and Zn use efficiency were determined by the method suggested by Moll (1982).

Results

Analysis of variance

Mean squares due to genotypes were significant for all the characters except for spikelets per spike. Therefore spikelet per spike was dropped from further analysis (Table1). Significant differences due to genotypes for various traits indicated that there was considerable variation among the genotypes. Genotype × fertilizer (G × F) interaction was significant for majority of the characters in *T. aestivum, T. durum*, triticale and synthetics. This indicated that genotypes differed in their response from low to optimum input conditions for the characters under study.

Mean performance of genotypes for various traits under low and optimum input conditions

The varieties LoK1 and HD2687 were having highest grain yield under low and optimum input conditions respectively, while HD 2687 showed maximum percent of increase over low input conditions. PBW343 and P7307 were having highest harvest index under low and optimum input conditions respectively, whereas Syn5 was found to show highest percent of increase over low input conditions (Table 2). Nitrogen content in grains was found highest in triticale genotypes TL2963 (2.43%) and TL2967 (2.74%) under low and optimum input conditions, respectively, while percent of increase over low input conditions was found to be highest in HD2687 (285.96%). For phosphorous content in grains TL2966

(0.52) and Syn36 (0.42) was found to be having highest P content under low and optimum input conditions, while percent of increase over low input conditions was found to be highest in TL2969 (69.30). Triticale genotype TL2963 (97.03) and (100.64) was observed to have highest Zn content under low and optimum input conditions, respectively, while percent of increase over low input conditions was found to be highest in P7531 (63.90). Genotypes Lok1 (25.43) and PBW343 (38.95) performed better for nutrient use efficiency under low and optimum input conditions, respectively, while for percent of increase over low input conditions was found to be highest in TL2967 (141.98). Genotypes PBW343 (97.38) and Lok1 (127.15) performed better in both the field conditions i.e. low and optimum input conditions, respectively, while TL2969 (62.67) responded better upon fertilizer application. For zinc use efficiency genotypes PBW343 (233.72) and Lok1 (381.46) were best performing under low and optimum input conditions, respectively, while TL2969 (103.38) responded better over low input conditions among all the genotypes.

Discussion

The increase in mean performance of grain yield from low to optimum input conditions was up to 75.30% in *T. durum* group followed by triticale group (68.3%), Synthetic wheat group (67.1%) and *T. aestivum* group (56.80%). The higher increase under optimum input conditions indicated the potential for fertilizer responsiveness of the genotypes which can be used in breeding programme for improvement of the trait under consideration. With regard to responsive genotypes in various groups the genotypes HD2687 in *T. aestivum* group, P7531 in *T. durum* group and TL2967 in triticale group and Syn 5 in synthetic wheat group were highly fertilizer responsive for grain yield. Singh and Prasad (1998) indicated that N application (0-80 kg N /ha) significantly increased the grain yield of wheat. Azad *et al*., (1998) found significant increase in yield of wheat due to increase in rate of fertilizer application from 100 percent recommended dose of NPK to 150 percent. Genotypes for harvest index responded from low to optimum input conditions upto 32.57% in *T. durum* group followed by 32.320% in synthetic wheat group, 29.50 % in triticale group and 22.41 % in *T. aestivum* group. Responsiveness of WH 1021 in *T. aestivum* group, HI8498 in *T. durum* group, TL 2968 in triticale group and Syn 36 in synthetic wheat group was high for biological yield, while for harvest index, DBW 17 in *T. aestivum* group, P7531 in *T. durum* group, TL 2967 in triticale group were highly responsive genotypes from low to optimum input conditions. Similarly Torabi and Malakuti (1997) found that the application of N (0-80 kg N/ha) increased grain yield but decreased harvest index of wheat.

The increase in mean performance of genotypes for N content in grams in various groups from low to optimum input conditions was upto 121.96% in *T. aestivum* group followed by 33.81% in *T. durum* group followed by 30.36% in triticale group followed 26.62% in synthetic wheat group. Similarly the mean performance of the genotypes for P content in grams from low to optimum input conditions in various groups was upto 20.48% in *T. aestivum* group followed by 8.39% in synthetic wheat group followed by 5.26% in *T. durum* group followed by 3.12% in triticale group. With regard to responsiveness the genotypes HD 2687 in *T. aestivum* group, HI 8498 in *T. durum* group, TL 2968 in triticale group and Syn 5 in synthetic wheat group were highly responsive for N content in grains. For P content in grains the genotypes WH 1021 in *T. aestivum* group, WHD 943 in *T. durum* group, TL 2969 in triticale group, Syn 27 in synthetic wheat group were highly responsive. The mean performance of the genotypes for Zn content in grains showed high response from low to optimum input conditions in various groups. The genotypes Lok1 in *T. aestivum* group, P 7531 in *T. durum* group, TL 2968 in triticale group and Syn 24 in synthetic wheat group were highly responsive. Rengel and Graham (1995) observed that zinc may be important for an early establishment of crops on low fertility soil and also for high grain yield and concluded that crops grown from seed containing higher Zn content have distinct advantages which culminate in grater yield when grown in soil of low Zn status.

Table 1. Mean squares for various characters of wheat genotypes evaluated under Low and optimum input conditions

Grain yield	Replication (2)†	Fertilizer(F) (1)	Error(a) (2)	Genotype(G) (7)	G X F (7)	Error (b) (28)
T. aestivum	1.44	1062.58*	5.28	58.75*	17.82*	3.18
T. durum	3.06	1543.15*	4.85	47.82*	16.03	5.42
Triticale	19.89	740.49*	7.36	22.71	24.45*	5.60
Synthetic wheat	3.64	322.51*	0.71	15.85*	4.02	1.85
Biological yield per plant						
T. aestivum	20.33	2898.92*	10.05	307.36*	177.70*	12.58
T. durum	17.37	3584.53*	22.86	259.10*	162.54*	11.42
Triticale	148.61	1699.37*	77.85	190.23*	164.38*	41.45
Synthetic wheat	59.26	938.99*	4.82	168.57*	35.58	20.52
Nitrogen content in grain						
T.aestivum	>0.01	13.38*	0.01	0.14*	0.10*	0.06
T.durum	0.01	2.72*	0.05	0.34*	0.18*	0.03
Triticale	0.01	4.09*	0.02	0.55*	0.20*	0.01
Synthetic wheat	0.01	1.94*	>0.01	0.22*	0.33*	>0.01
Phosphorous content in grain						
T.aestivum	>0.01	0.09*	>0.01	0.03*	0.01*	>0.01
T.durum	>0.01	0.02*	>0.01	0.01*	0.02*	>0.01
Triticale	>0.01	>0.01	>0.01	0.03*	0.01*	>0.01
Synthetic wheat	>0.01	0.03*	>0.01	0.03*	0.01*	>0.01
Zinc content in grain						
T.aestivum	34.31	1914.85*	64.31	98.45*	24.46*	>0.01
T.durum	34.31	2167.72*	64.31	67.23*	22.52*	>0.01
Triticale	34.31	1040.06*	3.99	31.89*	34.35*	5.67
Synthetic wheat	1.94	381.10*	7.92	533.49*	5.65*	>0.01
Nitrogen use efficiency						
T.aestivum	473.74	533314.77*	2035.30	27680.89*	8937.17*	1631
T.durum	1512.79	762110.91*	2398.31	23633.10*	7917.26*	2674.92
Triticale	9811.03	365640.71*	3639.32	11211.18*	12074.28*	2764.71
Synthetic wheat	34.50	450.80*	64.10	22.13*	1985.73	915.07
Phosphorous use efficiency						
T.aestivum	9.19	3665.73*	79.13	1289.16*	364.13*	>0.01
T.durum	9.16	1453.10*	78.76	811.17*	253.92*	>0.01
Triticale	9.20	1381.38*	79.40	383.61*	413.95*	>0.01
Synthetic wheat	9.19	679.51*	79.19	298.33*	89.35*	>0.01
Zinc use efficiency						
T.aestivum	31.44	121962.46*	>0.01	10002.86*	3343.72*	>0.01
T.durum	31.28	76632.09*	120.70	6169.46*	2147.48*	>0.01
Triticale	31.44	53243.10*	120.54	3017.97*	3238.95*	>0.01
Synthetic wheat	31.15	24838.45*	120.99	2325.27*	827.47*	>0.01

*, **: significant at 5% and 1% level of significance respectively.

Table 2. Mean performance of genotypes for various traits under low and optimum input conditions

Sr. no.	Trait	Input conditions	Genotype	Mean	% of increase over low input conditions
1	Grain yield per plant (g)	Low input	Lok1	**21.45***	HD2687 (143.20)
		Optimum Input	HD2687	**31.49***	
2	Biological yield (g)	Low input	WH147	**75.30***	WH1021 (72.10)
		Optimum Input	PBW343	**91.10***	
3	Harvest index	Low input	PBW343	**32.50***	Syn5 (94.30)
		Optimum Input	P7307	**38.60***	
4	N content	Low input	TL2963	2.43*	HD2687 (285.96)
		Optimum Input	TL2967	2.74*	
5	P content	Low input	TL2966	0.52*	TL2969 (69.30)
		Optimum Input	Syn36	0.42*	
6	Zn content	Low input	TL2963	97.03*	P7531 (63.90)
		Optimum Input	TL2963	100.64*	
7	Nutrient use efficiency	Low input	Lok1	25.43*	TL2967 (141.98)
		Optimum Input	PBW343	38.95*	
8	Phosphorous use efficiency	Low input	PBW343	97.38*	TL2969 (62.67)
		Optimum Input	Lok1	127.15*	
9	Zinc use efficiency	Low input	PBW343	233.72*	TL2969 (103.38)
		Optimum Input	Lok1	381.46*	

*, **: Significant at 5% and 1% level of significance respectively.

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In Turkey, 10 million tons of wheat was produced in 1923 (Gokgol 1939).

This result was in agreement with result of Sahin and Yildirim (2004).

Similar effect has been widely studied prior to this study (Eser 1991; Bagci et al. 1995; Uzun and Yol 2013).

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References

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Journal article:

Toker C (1998). Adaptation of kabuli chickpeas (Cicer arietinum L.) to the low and high lands in the West Mediterranean region of Turkey. Turk J Field Crops 3:10-15.

Toker C and Canci H (2003). Selection of chickpea (Cicer arietinum L.) genotypes for resistance to ascochyta blight [Ascochyta rabiei (Pass.) Labr.], yield and yield criteria. Turk J Agric For27: 277-283.

Toker C, Canci H and Ceylan FO (2006). Estimation of outcrossing rate in chickpea (Cicer arietinum L.) sown in autumn.Euphytica 151: 201-205.

Article by Digital Object Identifier (DOI) number:

Yasar M, Ceylan FO, Ikten C and Toker C (2013). Comparison of expressivity and penetrance of the double podding trait and yield components based on reciprocal crosses of kabuli and desi chickpeas (Cicer arietinumL.). Euphytica doi:10.1007/s001090000086

Book:

Toker C (2014). Yemeklik Baklagiller. BISAB, Ankara.

Book chapter:

Toker C, Lluch C, Tejera NA, Serraj R and Siddique KHM (2007) Abiotic stresses. In: Chickpea Breeding and Management,YadavSS, Redden B, Chen W and Sharma B (eds.),CAB Int. Wallingford, pp: 474-496.

Online document:

FAOSTAT J (2013) http://faostat.fao.org/site/567/default.aspx#ancor. Accessed 15 May 2013.

Dissertation (Thesis):

Yasar M (2012). Penetrance and expressivity of double podding characteristic in chickpea (Cicer arietinum L.). Dissertation, Akdeniz University, Antalya.

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Abbreviations

Abbreviations should be defined at first mention and used consistently.

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