

GENOTYPE BY ENVIRONMENT INTERACTION AND PHENOTYPIC STABILITY OF YIELD AND QUALITY IN BROCCOLI (*Barassica oleracea* VAR. ITALICA).

Abd El-Hamed, Kh. E. and M. W. M. Elwan

Department of Horticulture, Faculty of Agriculture, Suez Canal University, 41522, Ismailia, Egypt.

ABSTRACT

Better understanding of genotype and environment interaction will help to optimize yield and quality of crops. The objective of this study was to partition the phenotypic variance of yield and quality traits in broccoli into component sources associated with genotype, environment and genotype by environment interaction. In addition, compare the patterns of stability across environments. Three broccoli genotypes have been evaluated in three different growing seasons for yield and both nitrate and vitamin C content. The results revealed a phenotypic variation in all studied traits among broccoli genotypes. A greater proportion of the phenotypic variation was associated with differences among environments. Analysis of variance uncovered a significant effect of genotypes for yield and chemical quality traits which indicates the existence of a high degree of genetic variability in the tested genotypes. Genotype by environment interaction was significant for yield and chemical quality traits indicating that these traits are modified to different levels by the environments where they grown and emphasis on the need for testing genotypes in multiple environments to obtain reliable results. The stability analysis revealed that different degrees of stability are existed among genotypes for yield and chemical quality.

Keywords: Broccoli, yield, quality, nitrate content, vitamin C, genotype by environment interaction, stability analysis.

INTRODUCTION

Genotype-by-Environment Interaction:

The plant genotype and the growing environment interact to produce an array of phenotypes. Genotype by environment interaction is the failure of differences between genotypes to be the same in all environments due to the inconsistencies in the relative performance of genotypes tested in different environments (Baker, 1988). Genotype by environment interaction is a major concern when developing economically important genotypes with wide geographical usefulness. A large genotype by environment interaction reflects the need for testing cultivars in multiple environments to obtain reliable results (Lynch and Walsh, 1998).

Nature and Causes of Genotype-by-Environment Interaction:

The ability of a single genotype to generate different phenotypes in disparate environments is termed phenotypic plasticity, which reflects the interaction between genotype and environment on developmental processes (Bradshaw, 1965; Sultan, 2000). Any individual organism is able to alter its morphology and/or physiology in response to changes in environmental conditions (Schlichting, 1986). Different specific phenotypic response systems can be found in plants in relation to different fluctuating stresses

(Bradshaw and Hardwick, 1989). It was suggested that phenotypic plasticity is under genetic control (Bradshaw, 1965; Schlichting, 1986). Two different hypotheses of genetic control of phenotypic plasticity have been proposed; allelic sensitivity and regulatory control (Via *et al.*, 1995).

Genotype by environment interaction is an important concern in plant breeding programs because it provides information concerning the adaptability of a specific genotype to an array of environments (Allard and Bradshaw, 1964; Hill, 1975; Weber and Wricke, 1990; Kang, 1990; Ceccarelli *et al.*, 1994). Adaptability is the genotype's ability to cope with environmental fluctuation (Romagosa and Fox, 1993). Broad adaptability means that a genotype will perform consistently across environments. The higher the proportion of the phenotypic variation attributed to the genotype by environment interaction for a particular trait, the lower the adaptability of that trait across environments. In addition, this information is useful for allocating crop improvement resources and in determining the most efficient breeding strategy since significant genotype by environment interaction suggests that multiple trials are required to assess the performance of cultivars across environments (Kang, 2002).

Partitioning the total phenotypic variation for a trait into its component sources (genotype main effect, environment main effect, and genotype by environment interaction) by analysis of variance is crucial in determining crop improvement strategies (Simmonds, 1997; Hohls, 1995). The interactions between genotypes and environments contribute to the total variance, which can be estimated and tested for statistical significance (Chahal and Gosal, 2002). When a high proportion of the phenotypic variance for a specific trait is due to genotypic differences, the greater the feasibility of genetic manipulation to improve trait performance. In contrast, if most of the phenotypic variance for the trait is associated with the environment, then cultural practices and crop management strategies might be employed to create growing conditions that favor improved trait performance. When a high proportion of phenotypic variance is described by genotype by environment interactions, then the most relevant approach would require genetic selection for the trait at specific locations or growing conditions. Partitioning of variance requires evaluating performance of genotypes in a range of environments. While the analysis of variance can be used to partition the total variance into its components due to main effects and interactions, it is not robust in the analysis of the genotype by environment interaction. since it does not help determining the actual response of individual genotypes to the diverse environmental factors (Chahal and Gosal, 2002) and fails to determine the pattern of response of genotypes to environments (Alberts, 2004).

Stability Analysis:

One of the methodologies of dealing with genotype by environment interaction is studying stability of the performance across multiple environments by analyzing and interpreting genotypic and environmental differences that can identify genotypes with consistent performance (for review see Lin *et al.*, 1986; Alberts, 2004). Stability analysis provides a general summary of the response patterns of genotypes to environmental change (Alberts, 2004). The goal is to search for stable genotypes that

perform relatively the same over a wide range of environments and show desirable performance levels of the studied trait for use in commercial production or classical breeding programs.

Several stability parameters have been proposed (Freeman, 1973; Lin *et al.*, 1986; Lin and Binns, 1994). Francis and Kannenberg, (1978) suggested a simple description method for grouping genotypes on the basis of yield and consistency of performance represented by the coefficient of variation (CV). The mean-CV method was designed primarily to aid in studies on the physiological basis for yield stability. It represents a simple descriptive method for grouping genotypes from yield data collected over several environments (Francis and Kannenberg, 1978). Genotypes that show low CV across a wide range of environment are said to be well buffered since they display a great deal of stability as they tested across diverse environments. Genotypes that combine high and stable yield are the most desirable ones. The mean-CV method has been used in several studies to access yield stability in different crops (Asante and Dixon, 2002; Abdulai *et al.*, 2007).

Another simple measure of phenotypic stability was suggested earlier by Lewis (1954) who expressed stability factor as the genotype performance in high yielding environment proportional to the genotype performance in low yielding environment. When stability factor equals one, indicates the maximum phenotypic stability because yield of the genotype remains the same in both high and low yielding environments and the greater the deviation of stability factor from unity the less stable is the genotype (Chahal and Gosal, 2002).

Broccoli Yield:

Crop improvement for specific traits in traditional breeding programs depends on the existence of genetic variation that can be accessed through sexual hybridization and selection. Significant variation for yield has been reported for broccoli (Kostewicz, 1984; Cszinszky and Jones, 1983; Tan *et al.*, 1999a; Kalia *et al.*, 2005). The observed variation suggests that available germplasm can be utilized in a breeding program to develop new genotypes with enhanced yield. Most of the previous reported variation has been observed in a single environment which does not allow for the estimation of the role that the environment has on the phenotypic expression of yield.

Testing genotypes across environments is necessary to partition the observed variability into components associated with the genotype, environment, and genotype by environment interaction. Few studies have investigated the effect of genotype by environment interaction for yield in broccoli. Environment as a source of variation was highly significant and was the most important factor contributing to the total yield variation (Shattuck *et al.*, 1986). Both genotype and genotype by environment interaction were significant factors even though they contributed much less percentages to the total variation (Shattuck *et al.*, 1986). In another study by Antonova *et al.* (2010), the variation associated with genotypes represented a higher proportion of total variation (approx. 36%) while the environmental effect remains the most important factor.

The pervious reports suggest that while the environment exerts huge effect on determine broccoli yield, genetic regulation play a significant role.

The significant genotype by environment interaction observed in these studies indicated the need for testing genotypes in multiple environments to obtain reliable results particularly when developing genotypes with wide geographical usefulness (Kang, 1998).

Broccoli Quality:

In the present market economy, product quality has become increasingly important. Broccoli is a rich source of vitamin C. The content of vitamin C can be influenced by various factors such as genotypic differences, growing season and cultural practices (Lee and Kader, 2000). Nitrogen fertilizers, especially at high rates seem to decrease the content of vitamin C in many fruits and vegetables including broccoli (Eheart, 1966) although other results have been reported (Vallejo *et al.*, 2003).

High dietary intake due to the high nitrate content of certain vegetables has generated concern about the possible health effects. The toxicity of nitrate per se is low, but in humans 5-10% of the ingested nitrate is converted into the more toxic nitrite by gastrointestinal reduction (Boink and Speijers, 2001). Although earlier reports linking nitrate with the occurrence of cancer are largely un-substantiated, other nitrate-induced syndromes, such as methaemoglobinemia in infants (blue baby syndrome) have been confirmed (Addisoot and Benjamin, 2004; Fewtrell, 2004).

The present study was conducted to provide information on the extent of genotype, environment and genotype by environment interaction effects on broccoli yield and quality. Also, evaluate the stability of the studied traits in three broccoli cultivars.

MATERIALS AND METHODS

Plant Material and Growth Conditions:

Field experiment was conducted at the Experimental Research Farm, Faculty of Agriculture, Suez Canal University, Ismailia, Egypt. The experiment was carried out in spring and falls of 2008/2009 and was repeated in fall of 2009/2010. The soil of the experimental site was sandy soil (85.21% sand, 11.5% silt and 3.29% clay) with pH 8.27 and EC 0.47 dsm⁻¹. Before each planting, the experimental location was prepared three months before transplanting. During preparation, a rate of 20 m³ of cattle manure plus 300 kg calcium superphosphate (15.5 % P₂O₅) per feddan were supplemented, then the soil of the site was cleared, ploughed, harrowed and divided into plots.

The experiment was laid-out in a randomized complete block design with at least three replicates. Broccoli genotypes were "Sultan F1" (Asgrow Seed Company, USA), "Majestic F1" (Sakata Seed America Inc., USA) and "Marathon F1" (Sakata Seed America Inc., USA). Each cultivar occupied six rows per replicate, each row 10 m in length and 0.6 m in width containing fourty plants at a spacing of 0.4 m within the row.

Seeds of broccoli cultivars were sown in 209-cell styropham trays under greenhouse conditions. The trays were filled with a soil mixture (peat and vermiculite mixes in 1:1 v/v, enriched with different nutrients). After emerging, they were watered with a commercial nutrient solution (19-19-19

N-P-K with micronutrient) at a dilution of 1:200. The seedlings were maintained under high humidity and with day/night temperature of 29.8 °C for fall-winter season and 25.9 °C for spring season, for four weeks. In spring season, Broccoli seedlings, four weeks old, were planted on the third top of slope ridges, from the mid of February to the end of May for spring. With respect to fall-winter seasons, broccoli seedlings were planted from the end of October to the mid of February. Nitrogen fertilizer (90 kg N/fed.) was added at three equal doses 4, 6 and 8 weeks after transplanting. Recommended practices for disease and insect control were followed.

Data Collection and Chemical Analysis:

Fresh weights of curds at commercial fresh market maturity were recorded three times per week on all plants in the experimental unit starting April 22, 24 and May 5 (spring season) and January 5, 11 and 20 (fall season), for "Sultan F1", "Majestic F1" and "Marathon F1", respectively. Fresh weights of lateral curds were recorded one time per season on three plants per row (eighteen per experimental unit), after two weeks of the harvest of central curds. Central and lateral curds were harvested when they reached marketable size (depend on the genotype). Total yield was determined by summing up weight of produced central curds together with produced lateral curds. The extraction and determination of ascorbate (Vitamin C) was performed using the protocol of Pearson (1970) by titration method using 2,6 dichlorophenolindophenol.

During both growing seasons, approx. 100 g of florets from four broccoli heads (flower buds and second stem branching) were placed in a forced-air oven at 70 °C for 72 h. Dried samples were ground in a Wiley mill to pass through a 20-mesh screen and analyzed for nitrate content. Nitrate content of curds was determined spectrophotometrically at 540 nm, then calculated as mg/kg dry weight as described by Singh (1988).

Statistical Analysis:

Analysis of variance (ANOVA) and partitioning of variance components were conducted using the software package Statistica™ for windows. ANOVA was used to analyze the effects of genotype, environment and genotype by environment interaction on total phenotypic variation. Analysis of variance was performed using the linear model (Kuehl, 2000): $\chi_{ij} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \varepsilon_{ij}$, Where:

χ_{ij} = phenotypic value of the i genotype in environment j

μ = the overall mean

α_i = the fixed effect of genotype i

β_j = the random effect of environment j

$(\alpha\beta)_{ij}$ = the random interaction effect of genotype i in environment j

ε_{ij} = the random experimental error associated with χ_{ij}

The stability of individual genotype's performance to the environment was analyzed using Lewis stability factor (Lewis, 1954). The equation to calculate the Lewis stability factor was mean H.E/ mean L.E where mean H.E is the mean value of a genotype in high yielding environment and mean L.E is the mean value of same genotype in low yielding environment. Coefficient of variation (CV) used in genotype grouping technique suggested by Francis and Kannenberg (1978) were calculated for each genotype according to the

equation: $\sigma/x * 100$ where σ is the standard deviation of each genotype and x is the mean value of the same genotype (Kuehl, 2000).

RESULTS

Climate conditions (air temperature and relative humidity)

Differences between the growing seasons in daily and night temperatures were observed (Figure 1a). During the fall-winter seasons, the daily temperature decreased from 25.93 °C to 22.1 °C in fall 2008-2009 and from 28.53 to 27.8 in fall 2009-2010 throughout the first month of transplanting (November). Also the night temperature followed the same trend, whereas, it decreased from 16.7 °C to 14 °C in fall 2008-2009 and from 11.23 °C to 10.64 in fall 2009-2010. However, the daily and night temperatures increased from the mid of February to the mid of March (18.39 °C to 26.75 °C and from 6.98 °C to 12.9 °C, respectively) for spring season (first month of transplanting). Values of daily and night temperatures in fall-winter seasons were still higher than spring season during the first month of cultivation. Daily and night temperatures increased in spring season reaching 31.8 °C and 16.13 °C, respectively, on the otherhand, daily temperature decreased to 19.7 °C in fall-winter season of 2008-2009 and 21.87 in fall-winter season of 2009-2010. Regarding the relative humidity, considerable differences between spring and fall-winter seasons (Figure 1b) were observed only in the middle of the season (between the fourth and tenth week).

Broccoli Yield:

The yield of the three broccoli genotypes over all the three environment study period are summarized in Table (1). In general, the data showed considerable environmental and genotypic variation. The total yield of genotypes ranged from 5.77 to 8.28 t/fed while it was from 3.68 to 7.2 t/fed for central curd yield and finally 1.1 to 2.1 t/fed for lateral curd yield. When the genotypes were ranked for total yield, there was partial agreement from environment to environment indicating the importance of genotype in determining this character. "Marathon F1" and "Sultan F1" usually exhibited the highest and lowest central curd and total yield, respectively. The three environments mean total yield performances of these two genotypes were 112 and 78% of the grand mean while the percentages were 126 and 64 % for the central curd yield respectively. Later-maturing genotypes consistently averaged greater yields. In environment no. 2 (fall-winter season of 2008/2009) the genotypes had a significantly higher mean central curd and total yield than other environments (both spring and fall-winter season 2009/2010) (Table 1).

The pooled analysis of variance over environments for 3 growing seasons is presented in table (2). Analysis of variance was applied to partition the total phenotypic variation for studied traits among the three broccoli genotypes into components associated with genotype (G), environment (E), and genotype by environment interaction (G x E).

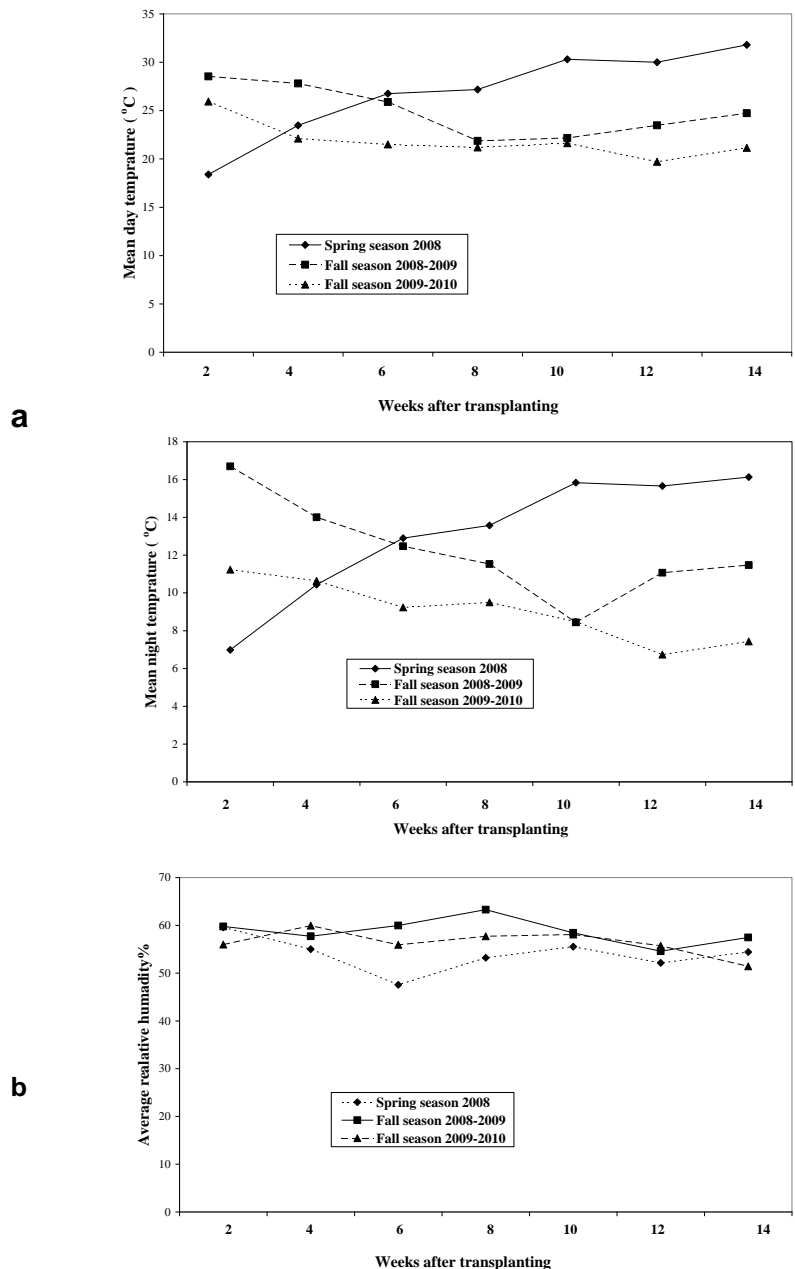


Figure 1: Daily and night (a) air temperatures as well as air relative humidity (b) recorded in the region of Ismailia during the spring season 2008 as well as fall-winter seasons of 2008-2009 and 2009-2010.

Table (1): Yield and chemical quality traits of three broccoli genotypes grown over three environments.

| | Environment 1 (spring 2008) | Environment 2 (fall 2008-2009) | Environment 3 (fall 2009-2010) | Mean |
|--|--------------------------------|-----------------------------------|-----------------------------------|----------|
| Central curd yield (t/fed.) | | | | |
| Sultan F1 | 3.59 fg | 4.35 e | 3.10 g | 3.68 c |
| Majestic F1 | 3.83 f | 8.75 a | 6.39 c | 6.32 b |
| Marathon F1 | 4.89 d | 9.21 a | 7.48 b | 7.19 a |
| Mean | 4.10 c | 7.44 a | 5.66 b | 5.73 |
| Lateral curd yield (t/fed.) | | | | |
| Sultan F1 | 1.98 b | 3.05 a | 1.28 de | 2.10 a |
| Majestic F1 | 1.07 ef | 2.89 a | 1.56 cd | 1.84 b |
| Marathon F1 | 0.80 fg | 0.61 g | 1.91 bc | 1.10 c |
| Mean | 1.28 c | 2.18 a | 1.58 b | 1.68 |
| Total yield (t/fed.) | | | | |
| Sultan F1 | 5.56 d | 7.39 c | 4.38 e | 5.78 b |
| Majestic F1 | 4.90 de | 11.64 a | 7.95 c | 8.16 a |
| Marathon F1 | 5.69 d | 9.82 b | 9.38 b | 8.30 a |
| Mean | 5.38 c | 9.62 a | 7.24 b | 7.41 |
| Nitrate content (mg/kg DW) | | | | |
| Sultan F1 | 112.65 | 26.09 | 55.92 | 64.89 |
| Majestic F1 | 58.73 | 35.95 | 35.23 | 43.30 |
| Marathon F1 | 119.59 | 56.33 | 95.95 | 90.62 |
| Mean | 96.99 | 39.46 | 62.37 | 66.27 |
| Vitamin C content (mg/100 g FW) | | | | |
| Sultan F1 | 147.90 c | 152.26 c | 123.2 e | 141.12 b |
| Majestic F1 | 151.10 c | 163.54 ab | 147.02 cd | 153.89 a |
| Marathon F1 | 156.25 bc | 168.92 a | 140.73 d | 155.30 a |
| Mean | 151.75 b | 161.57 a | 136.98 c | 150.10 |

Values are the means of at least three replicates. Values followed by the same letter within a column are not significantly different at the 0.05% level of probability according to Duncan's multiple range test.

For total yield, environmental differences described a great percent of the variation in the model. Mean squares due to environmental effects were significant and environmental sum of squares accounted for almost 55%, 39%, and 22% of total sum of squares in the model for central curd, lateral curd and total yield respectively (Table 3). Variation due to genotype was significant and represented the second most important factor contributing to the total variation for yield (Table 3). Variation affiliated with genotype by environment interaction was found to be low although significant for all yield components. Genotype by environment interaction was significant for the three yield components indicating that this trait in the genotypes was modified to different extents by the environment in which they were grown. The interaction between genotype and environment was further evaluated by two stability parameters (Table 4 & 5).

Genotype "Sultan F1" possessed the lowest central, lateral and total yield coefficient of variation and stability factor among other genotypes. In contrast "Majestic F1" showed the highest degree of un-stability for total yield in both stability parameters suggesting sensitivity to environmental variation.

“Marathon F1” exhibited average total yield stability responses under the varying environments encountered in this study.

Table (2): Analysis of variance for yield and chemical quality traits in three broccoli genotypes grown over three environments.

| | df | SS | MS | P |
|---------------------------|----|---------|---------|--------------|
| Central curd yield | | | | |
| Environment (E) | 2 | 2164.49 | 1082.24 | 0.000000 *** |
| Genotype (G) | 2 | 2585.78 | 1292.89 | 0.000000 *** |
| G*E | 4 | 802.09 | 200.52 | 0.000000 *** |
| Lateral curd yield | | | | |
| Environment (E) | 2 | 155.15 | 77.58 | 0.000000 *** |
| Genotype (G) | 2 | 205.64 | 102.82 | 0.000000 *** |
| G*E | 4 | 348.35 | 87.09 | 0.000000 *** |
| Total yield | | | | |
| Environment (E) | 2 | 3468.00 | 1734.00 | 0.000000 *** |
| Genotype (G) | 2 | 1495.07 | 747.53 | 0.000000 *** |
| G*E | 4 | 1312.19 | 328.05 | 0.000000 *** |
| Nitrate content | | | | |
| Environment (E) | 2 | 77988.7 | 38994.3 | 0.000000 *** |
| Genotype (G) | 2 | 51302.0 | 25651.0 | 0.000000 *** |
| G*E | 4 | 17307.4 | 4326.8 | 0.000000 *** |
| Vitamin C content | | | | |
| Environment (E) | 2 | 15034 | 7517 | 0.000000 *** |
| Genotype (G) | 2 | 7611 | 3806 | 0.000000 *** |
| G*E | 4 | 2145 | 536 | 0.023950 * |

df = degrees of freedom, SS= Sum of Squares, MS= Mean Squares and P= Probability
* and ***, significant at 5% and 0.1% level, respectively.

Table (3): Percentages of total phenotypic variation of yield and chemical quality traits associated with genotype, environment, and genotype by environment interaction for three broccoli genotypes grown over three environments

| | Genotype (G) | Environment (E) | G*E |
|--------------------|--------------|-----------------|------|
| Central curd yield | 46.6 | 39.0 | 14.4 |
| Lateral curd yield | 29.0 | 21.9 | 49.1 |
| Total yield | 23.8 | 55.3 | 20.9 |
| Nitrate content | 35.0 | 53.2 | 11.8 |
| Vitamin C content | 30.7 | 60.7 | 8.6 |

G*E= Genotype by environment interaction

Table (4): Mean and coefficient of variation of yield and chemical quality traits for three broccoli genotypes grown over three environments.

| | Sultan F1 | | Majestic F1 | | Marathon F1 | |
|---------------------------------|-----------|------|-------------|------|-------------|------|
| | X | CV | X | CV | X | CV |
| Central curd yield (t/fed.) | 3.68 | 21.2 | 6.31 | 34.7 | 7.2 | 29.3 |
| Lateral curd yield (t/fed.) | 2.1 | 36.6 | 1.84 | 60.9 | 1.1 | 74.0 |
| Total yield (t/fed.) | 5.77 | 25.1 | 8.16 | 37.8 | 8.28 | 29.1 |
| Nitrate (mg/kg DW) | 64.9 | 66.7 | 43.3 | 35.8 | 90.6 | 42.2 |
| Vitamin C content (mg/100 g FW) | 141.1 | 11.8 | 153.9 | 9.0 | 155.3 | 12.4 |

X= Mean and CV= Coefficient of Variation

Table (5): Lewis's stability factor for three broccoli genotypes measured for different yield and chemical quality traits.

| | Sultan F1 | Majestic F1 | Marathon F1 |
|--------------------|-----------|-------------|-------------|
| Central curd yield | 1.4 | 2.3 | 1.9 |
| Lateral curd yield | 2.4 | 2.7 | 3.2 |
| Total yield | 1.7 | 2.4 | 1.7 |
| Nitrate content | 4.3 | 1.7 | 2.1 |
| Vitamin C content | 1.2 | 1.1 | 1.2 |

For better judging and selection of genotypes, the genotypes grouping technique suggested by Francis and Kannenberg, (1978) were used by plotting individual genotype mean yield (Y-axis) against the coefficient of variation (CV) percent for each genotype (X-axis) (Figure 2). By drawing horizontal line through the genotype mean yield of 5.73, 1.67 and 7.4 t/fed for central, lateral and total yield respectively and a vertical line through the CV percent grand mean (28.4, 57.3 and 30.7 for the same yield components respectively), four quadrants were formed (Figure 2). Quadrant I represents the high yield, small variation (high stability), quadrant II represents the high yield, large variation (low stability), quadrant III represents low yield, small variation (high stability) and quadrant IV represents low yield, large variation (low stability). Based on these criteria, "Marathon F1" can be considered a stable genotype with high yielding which suggests that this genotype performed well in the entire environments under study. "Majestic F1" fall within quadrant II, so it considered less stable but with high yield, therefore, may be targeted to a specific environment where it may perform well.

Broccoli Quality:

Curd nitrate and vitamin C content averaged over replication from the three environments in the three studied broccoli genotypes are summarized in Table 1. Nitrate content mean ranged from 64.9 to 90.6 (mg/kg DW). "Marathon F1" recorded the highest mean value, while "Majestic F1" gave the lowest mean curd nitrate content. The three environment nitrate content mean of these two genotypes was 137% and 65% of the grand mean. Spring evaluation gave significantly higher nitrate content for all the three studied genotypes compare to both fall evaluation seasons. The combined analysis of variance is presented in Table 2. Effects of genotype, environment and genotype by environment interaction were significant and contributed 35%, 53%, and 12% to the total sum of squares, respectively (Table 3). Nitrate content response of the genotypes to environments was differential. In general, "Marathon F1" tends to accumulate more nitrates in curds however in the spring evaluation the content was 1.7-fold over the fall evaluation. "Sultan F1" possessed the lowest stability for nitrate content measured by stability factor and CV (Table 4, 5) (Figure 1). In contrast, "Majestic F1" showed the highest degree of stability for the same trait in both stability parameters (Table 4, 5) (Figure 2).

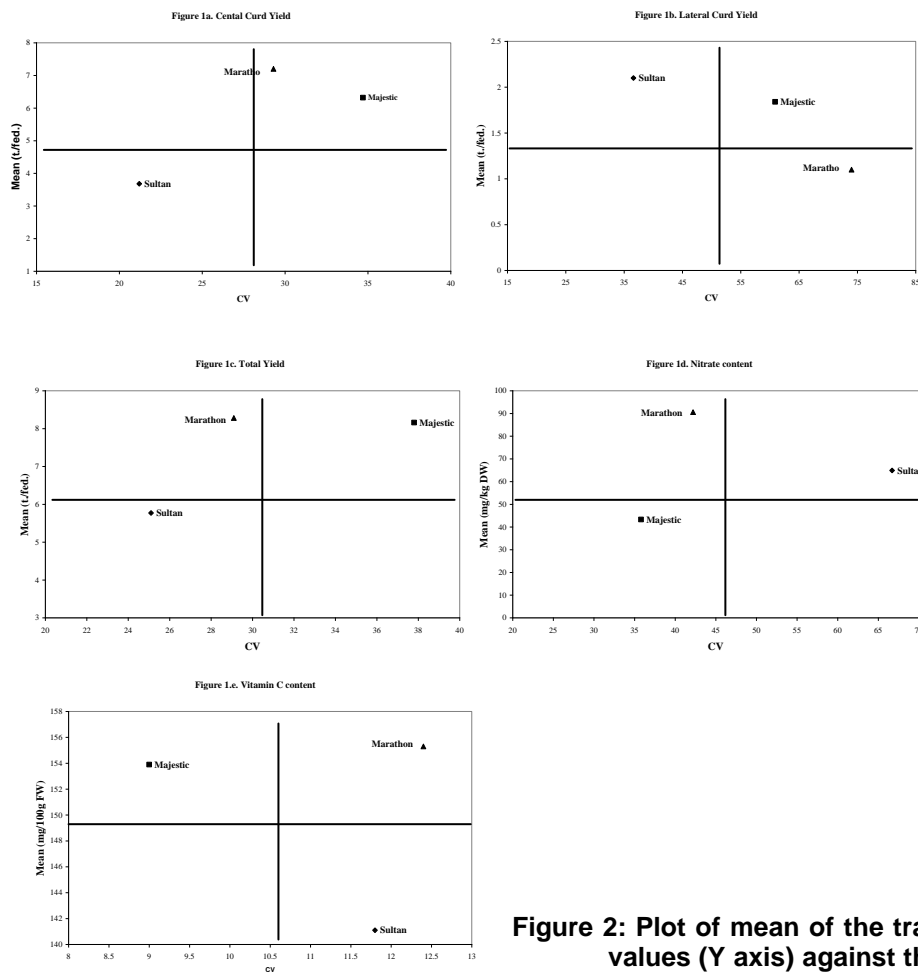


Figure 2: Plot of mean of the trait values (Y axis) against the coefficient of variation (X axis) for three broccoli genotypes.

The mean content of vitamin C for the three broccoli genotypes ranged from 155.3 to 141.1 (mg/100 FW) (Table 1). "Marathon F1" scored the highest mean value for vitamin C, while "Sultan F1" gave the lowest mean value. The three environments mean vitamin C content of these two genotypes was 103% and 94% of the grand mean. The response of vitamin C content to environments was contradicted. When the seasons were ranked for vitamin C content there was no clear trend. The fall-winter of 2008-2009 evaluation gave significantly higher vitamin C content than spring evaluation and also than the fall-winter of 2009-2010 evaluation. The combined analysis of variance is presented in Table 2. Both environmental and genotypic effects

were significant and accounted for most of the detected variation (61% and 31% of the total sum of squares respectively) (Table 3). Genotype by environment interaction was hardly significant and had a little contribution to the total variation (9%) (Table 3). "Marathon F1" tends to accumulate more vitamin C in curds than other genotypes although not significantly different than "Majestic F1". "Majestic F1" possessed the highest degree of stability for vitamin C measured by different stability parameters, while "Sultan F1" and "Marathon F1" showed a comparable level of stability for vitamin C in both stability parameters (Table 4, 5) (Figure 2).

DISCUSSION

Development of elite broccoli germplasm with enhanced yield and quality will potentially promote broccoli cultivation and production. The first step in a breeding program to optimize yield in broccoli is to partition variance into its component sources which requires evaluating performance of genotypes in a range of environments.

This investigation found that the phenotypic variation existing among broccoli genotypes for yield is primarily under environmental control. The observation that the greater proportion of the phenotypic variation is associated with differences among environments (Figure 1) tends to agree with the previous work concerning the expression of yield in broccoli (Shattuck *et al.*, 1986; Antonova *et al.*, 2010).

The significant environmental variation for yield that was observed among broccoli genotypes evaluated in fall and spring growing seasons are due to the fact that genotypes grow in the spring (harvested in the summer) and the fall evolution (harvested in the winter) would experience significantly different temperature regimes (Figure 1a), air relative humidity (Figure 1b), light intensities and quality, day-length, and rainfall which all have known to influence yield in plants (Tan *et al.*, 1999 a, b; Wurr *et al.*, 1991; Hardley and Pearson, 1998).

While the study took place in one location, the genotypes experienced substantially different growing conditions across the growing environments. Lower than optimal temperature (18.4 °C) at spring transplanting and higher than optimal temperature at maturity and harvesting (more than 30 °C) provides an environment distinct from that of fall planting (27.23 °C) and winter harvest (around 22 °C) (Figure 1a). Biotic stresses would also be unique to plants grown each season. All of these factors may have contributed individually or collectively to explain the greater contribution of the environment in determining yield of broccoli.

The analysis of variance revealed that the mean squares for genotypes were significant for total yield. This indicates the existence of a high degree of genetic variability in the material that can be exploited in a breeding programme which was also reflected in the broad ranges observed for total yield (Table 1). These results are confirmed by the previous reports by Tan *et al.*, 1999a ; Shattuck *et al.*, 1986; Antonova *et al.*, 2010, Kalia *et al.*, 2005).

The stability analysis identified stable genotypes for yield which may possess broad-range tolerance to both biotic and abiotic environmental stresses. The results of this investigation suggest that currently available commercial genotypes can be utilized in breeding programs to develop new broccoli germplasm with elevated yield. Stability differences detected among tested genotypes in this study emphasizes the need to survey over multiple environments (Piepho, 1996).

Since vegetables are major source of human diet, their chemical composition represents important quality characteristics influencing human health (Kushad, 2003). Both genetic and environmental factors influence the process of nitrate accumulation. This investigation revealed large genotypic differences in nitrate accumulation in broccoli (Table 1). For several vegetables, the same genotypic differences in nitrate accumulation have been reported (Reinink and Eenink, 1988). Environmental factors having an important effect on nitrate accumulation are light intensity, temperature, photoperiod, water supply and nitrogen supply (Maynard *et al.*, 1976). Since part of the ingested nitrate may be converted to nitrite or nitrosamines which may endanger the consumer's health, nitrate accumulation in edible parts of vegetable is a negative quality aspect from the nutritional standpoint. Apart from the effect of environmental factors on nitrate accumulation, this investigation also found a distinct variation between genotypes which is partially under genetic regulation as revealed from ANOVA analysis (Table 2).

Reinink and Groenwold (1987) found a high heritability estimates in various lettuce populations which suggest that genotypes with reduced nitrate content is possible by accumulating genes for low nitrate in these genotypes. However, the high environmental effects detected in this study suggested that the nitrate content of a specific genotype depends on environmental factors (Figure 1 & Table 2). Moreover, a significant interaction between genetic variation and environmental factors is existed in our results (Table 2) and by Reinink and Groenwold (1987). Not much work has been done on this interaction and the information is scarce. In order to select for cultivar with low nitrate content, more knowledge's on the interaction between different genotypes and environmental conditions are required (Blom-Zandstra, 1989).

More than 90% of the vitamin C in human diets is supplied by fruits and vegetables. Wide variation in the content of vitamin C among genotypes has been found (Lee and Kader, 2000; Vallejo *et al.*, 2003). This study found large genotypic differences in vitamin C between tested broccoli genotypes (Table 1). Environmental factors that have been reported to have effect on vitamin C include both pre-harvest and post-harvest factors (Lee and Kader, 2000). The ANOVA analysis showed that a substantial percentage of total variation is accounted for genetic differences between genotypes (Table 2). Vitamin C levels in eight broccoli genotypes were significantly affected by cultivar, season, fertilization and interaction between these factors (Vallejo *et al.*, 2003). Our results showed a similar trend as growing season represented the highest proportion of variation (Figure 1) and the interaction between genotypes and growing environments was significant although contributed less to the total variation. The considerable variation between broccoli

genotypes for vitamin C and the results of ANOVA analysis suggested the feasibility of developing elite broccoli genotypes with elevated vitamin C content.

In conclusion, although the high environmental regulation, our results support the feasibility of genetic manipulation of yield and chemical composition in broccoli. Extrapolation of the results obtained in this study to other broccoli genotypes and growing environments is constrained because of the low number of tested genotypes and because this investigation was conducted in a single location over multiple years.

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التفاعل بين التركيب الوراثي و البيئة المحيطة و الثبات المظهري للمحصول و جودة الرؤوس في محصول البروكولي

خالد السيد عبد الحميد و محمد وصفي محمد علوان

قسم البساتين- كلية الزراعة – جامعة قناة السويس – الاسماعيلية – جمهورية مصر العربية

ان الفهم الجيد للتفاعل بين التركيب الوراثي و البيئة سوف يساعد في زيادة و تعظيم محصول و جودة النباتات. الهدف من اجراء هذه التجربة هو تحديد مقدار الاختلافات المظهرية لصفات المحصول و الجودة في نباتات البروكولي المسئول عنها كل من التركيب الوراثي و البيئة و التفاعل بينهما بالإضافة الى دراسة الثبات الوراثي لهذة الصفات بين بيئة وأخرى.

و في هذه الدراسة تم زراعة ثلاث تراكيب وراثية (أصناف) من البروكولي و تم تقييمها لمدة ثلاث مواسم نمو مختلفة و ذلك لصفات المحصول و محتوى الرؤوس من النترات و فيتامين ج حيث أظهرت النتائج وجود تباينات في جميع الصفات تحت الدراسة بين أصناف البروكولي المختبرة وكان الجزء الأكبر من التباينات مرتبطا بالاختلافات بين البيئات التي نمت فيها الاصناف. و أظهر تحليل التباين تأثير معنوي للتركيب الوراثية على صفة محصول الرؤوس و محتواها الكيماوي و الذى يشير الى وجود درجة عالية من الاختلافات الوراثية بين الاصناف المختبرة مما يسمح بعمليات التحسين الوراثي و التربية لزيادة هذة الصفات. و أظهرت النتائج أيضا ان التفاعل بين التراكيب الوراثية و البيئة كان معنويا لكل الصفات المدروسة مما يشير الى أن هذة الصفات تستجيب بدرجات مختلفة للبيئة التي تنمو بها الاصناف مما يحتم تقييمها في عدد من البيئات لضمان الحصول على نتائج دقيقة. كذلك أظهر تحليل الثبات الوراثي أن الاصناف تمتلك درجات متباينة من الثبات لصفات المحصول و المحتوى من النترات و فيتامين ج.

قام بتحكيم البحث

**كلية الزراعة – جامعة المنصورة
كلية الزراعة – جامعة قناة السويس**

**أ.د / طه محمد السيد عمر الجزار
أ.د / فؤاد حسن محمد**