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# Influence of three soil moisture levels on early growth and proline content of some faba bean genotypes

**D. S. Darwish[1]*, S. A. Shrief[1], G. M. Fahmy[2] M. M.Y.Madany[2], A.M.Saleh[2] and Raad M. S. Al-Juboori[1]**

**[1] Agronomy Department, Faculty of Agriculture, Cairo University, 12613 Giza-Egypt.**
**[2]Botany and Microbiology Department, Faculty of Science, Cairo University, 12613 Giza- Egypt.**

**Abstract :** Ten faba bean (*Vicia faba* L.) genotypes were evaluated under three soil moisture levels in two pot trials during 2014/2015winter season. When the plants were 30 days old, they were subjected to three treatments of watering for a period of 20 days. The levels were 70% of the available soil-water capacity, AW (represented the normal irrigation; N), 45%, and 20% of AW (represented medium (M), and severe water stress (S), respectively). The objectives were to explore the extent of variation among faba bean genotypes in early growth stage under variable soil drought-prone conditions with different climatic factors.
Moisture levels were the most important source of variation for early growth parameters as well as leaflet RWC% and proline content of faba beans than genotypic differences or interactions. Combined analysis over both environmental conditions, proved that the accumulation of dry matter and its allocation in various organs of different faba bean varieties during the early growth stages and their tissues hydration and proline content depended greatly on climatic factors (particularly growing thermal units) and soil moisture. The rates of changes (regression coefficients) due to the reduction of AW were significantly negative for all traits except for Root/Shoot Dwt, relative root to plant Dwt and Proline content, which significantly increased as soil moisture decreased. This proved that the tested faba bean genotypes possessed intrinsic mechanisms of responses to water deficit during the early stage of growth.
Faba bean genotypes: M.1, M.3, C.4 and N.1 appeared to be the most sensitive ones since their lower RWCs under stress conditions accompanied to higher rates of RWC reductions. In spite of that cultivar C.5 shared C.4 in highest rate of RWC reduction (b=-0.72**), the first maintained significantly higher RWC (=55.71%). This may be due to the highest rate and content of increase proline (b=0.010** & 5.74 mg $g^{-1}$ fresh wt) of C.5, which is two folds as much as higher of C.4 (b=0.05**& 3.83 mg $g^{-1}$ fresh wt). Cultivars exhibited lower declines of RWC due stress corresponded to higher contents of moisture in the tissues of leaflet may be considered as tolerant to water stress (i.e G.843 and C. 49).
Our calculations revealed that the levels of proline of C.5 grown under medium (M) and water stress (S) conditions contributed 5.3% and 12.8% of the total osmotic potential. The percentages contribution of proline to the total predicted osmotic potential of the genotype C.5 indicates that this amino acid might play a role in the overall osmotic adjustment of the cells.
**Keyword:** *Vicia faba*, Genotypic variation, Watering regimes, Dry matter, G.D.D, RWC, Proline, Drought tolerance, Drought sensitive.

## Introduction

The major problem facing faba bean production is the instability and variations of its yield among seasons and locations, which referred to several biotic and abiotic limitations (Darwish and Abdalla,[1]). Faba bean is more sensitive to drought, salinity, and heat than other grain legumes (Amede and Schubert, [2]). According to the expected scenarios stated by the Intergovernmental Panel on Climate Change, the impacts of elevated global $CO_2$ and the associated changes in temperature and precipitation are likely affecting the plant life on the planet. The recurrence and severity of the drought conditions are expected resulting in a consequent declines and variability in the yields of different crops. Hitherto, developing, and breeding faba bean genotypes tolerant to the biotic and abiotic stresses, and characterized by high potential yields is an essential way to alleviate these effects(Darwish *et al.*,[3]; Darwish and Fahmy,[4]; Darwish,[5], Khan *et al.*,[6]; Siddiqui *et al.*,[7]). Under stress conditions, the plants develop a variety of morphological, reproductive, and physiological attributes to increase their tolerance (Blum,[8]). The osmotic adjustment (OA) is an important mechanism associated with drought tolerance. It involves the accumulation of solutes, like sugars, proline, and glycine betaine in the cells to decrease the osmotic potential, which is beneficial for the plant cell (Pérez-Pérez *et al.*,[9]). The concomitant occurrence of the attribute of OA together with water maintenance in the cells by regulation of transpiration possibly increases the tolerance of the plant to drought. AbouTaleb and Darwish,[10] reported that faba bean genotypes respond to drought differently, and their progenies were better able in some traits related to drought avoidance, such as stomatal size and density, which regulate both transpiration and photosynthesis.

The identification of plant responses to environmental stresses, such as salinity and drought, is usually performed through field trials and pot experiments. Rapid evaluation of numerous genotypes under field conditions is tedious, expensive, needs labor, farm equipments, and prolonged time. Both pot experiments and controlled environments have been applied to effectively induce water stress in faba beans and other leguminous crops (Amede *et al.*,[11], Ricciardi *et al.*,[12], Khan *et al.*,[13], Siddiqui *et al.*,[7]).

The objectives of the present investigations were to explore the extent of variation among some faba bean genotypes in early growth stage under variable soil drought-prone conditions and different climatic factors (environments).The elucidation of genotypic response of faba beans to soil moisture levels may be of great benefit for breeding programs.

## Materials and Methods

### Plant Material and Experimental Procedures

Ten faba bean genotypes comprised nine local improved varieties and exotic one (Luz-De from Turkey) were used. Five of the Egyptian varieties were kindly obtained from Food legumes Section, Field Crops Res. Institute and the other four varieties were provided by Agronomy Dept., Faculty of Agric., Cairo University. All varieties are medium seed size (Equina type) except Nubaria 1 (Egyptian) and Luz-De (Turkey) which are major seed type.

Two pot experiments were conducted during 2014/2015 winter season in natural conditions of the Experimental Farm, Agronomy Dept., Faculty of Agriculture, Cairo University, Giza, Egypt. Each trial was carried out as a Completely Randomized Design with three replications using two factors (faba bean genotypes and three moisture levels). The first trial was carried out from November 27, 2014 to January 15, 2015 and included the ten cultivars. However, the second one was dealt only with 5 (M.1, S.1, C.25, C.49 and Luz-DE) of the 10 cultivars and started in the $20^{th}$Jan 2015 for 50days also.

The experimental unit included one plastic pot (30 cm in diameter x 40 cm height), which filled with exactly 6500 g of the oven-dried soil. Five homogeneous healthy faba bean seeds were sown in each pot at 2 cm depth from soil surface. After emergence, the seedlings were thinned to 3 equally spaced individuals per pot. The used soil was obtained from the surface layer (0-30 cm depth) of the arable fields of the Experimental Farm. The soil was sieved through 2mm sieves to remove plant remains, gravels and stones, then air dried and finally placed in stainless steel trays for oven drying at 105˚ C for 48 hours.

Physical and chemical properties of the soil were sandy clay loam with pH= 7.92, EC= 0.58 $dsm^{-1}$, total souble anions (meq/l) = 7.6, total soluble cations (meq/l) =7.6 and $CaCo_3$ % = 4.2.The soil moisture at the field capacity was 40.2% oven dry soil, while the permanent wilting percentage was 20.1. The difference between the field capacity and the permanent wilting point is the available water capacity (AW).

During the first 30 days of each trial, the pots were irrigated with given volume to maintain 70% of AW as the optimal soil water conditions. When the plants were 30 days old, they were subjected to three moisture levels for a period of 20 days. The levels were: 70% of the AW (as normal, N treatment), 45%, and 20% of the AW as medium (M) and stressed (S) treatments, respectively. The water content of the soil was maintained by adding tap water every day to replace the water lost by evaporation or absorption by roots. The amount of water lost was determined by weighing the pots every day and the reduction of the soil water content of any treatment was added to hold the pot at its given moisture level.

### Plant Measurements

On the day $51^{th}$ day after the beginning of each experiments, or after 20 days from adoption of the watering treatments, the shoot length (cm), number of leaves, root and shoot dry of all plants per pot were recorded and those per plant were estimated. The dry matters were obtained after drying the fresh samples of roots and shoots in hot air oven at 70 C for 48 h. The mean leaflet area (LA), $cm^2$ was estimated according to Peksen[14] using leaflet length and width from the basal leaflet of the third unfolded fully expanded sun leaf (counting from the terminal bud).

The relative water content (RWC) of the leaf tissues was determined at the period from 10.00 to 12.00 h. using discs of the basal leaflet of the third unfolded fully expanded sun leaf (Barrs and Weatherly, [15]).

Free proline was determined in plants sampled at the same time of RWC according to the method of Bates et al. [16] from samples of leaflet tissues comparable with those used for RWC determinations. Fresh plant material (0.5 –1.0 g) was homogenized in 10 ml of 3% sulfo salicylic acid and the homogenate was filtered. The filtrate (2 ml) was treated with 2ml acid ninhydrin and 2 ml of glacial acetic acid, then with 4ml of toluene. Absorbance of the colored toluene solutions was read at 520 nm.

Regular analyses of variance of CRD of factorial experimental design were performed of obtained data of each trial in addition to plant dry weight, root/shoot dry weight(R/Sh) as well as relative root to plant dry weight (RRP Dwt). Combined analyses of variance over trials of the results of the same 5 genotypes were carried out after testing the homogeneity of error terms. The extents of phenotypic and genotypic variances over trials of each moisture level treatment were calculated using proper formulae of the expected mean squares partitioning.

## Results and Discussion

### Significance of Mean Squares

Mean squares due to moisture levels, 10 cultivars and their interactions were statistically significant for all studied traits except of genotypes for RWC (Table 1). The magnitudes of variance due to moisture levels (M.L) were much higher than those of cultivars (CVs) or their interactions for all studied traits. In this regard, variances of M.L ranged from two folds (of No. leaves and RDwt) to 40 folds (of RWC and proline) as much as higher of those by CVs. On the other hands, mean squares of Cvs x M.L were the least one for all tabulated traits except for RWC. This proved that, the investigated moisture levels were the most important source of variation for early growth parameters of faba beans than genotypic differences or interactions. However, using data of one trial not permit to extract the effects of environments and its interaction with the investigated factors. Thus, the utilization of common varieties' data in both pot trials will detect the environmental effects and its interactions with investigated factors (5 Cvs and 3 moisture levels). Combined analysis over environmental conditions (E) recorded that E, moisture levels (M.L) and CVs affected highly significantly all tabulated traits (Table 2) except E for shoot L and relative root /plant Dwt (RRP Dwt). The M.L x E and CVs x E interactions were highly significant for all traits except for shoot L in both interactions and RWC in CVs x E. This means that the investigated moisture regimes during the early growth stages of faba beans markedly affected dry matter accumulation and its allocations. These effects varied due to the dominated environmental conditions and the tested genotype. On other words, the accumulation of dry matter and its allocation in various organs of different faba bean varieties during the early growth stages depended greatly on climatic factors and soil moisture. The climate of the first trial which conducted during Nov., 27/014 to Jan., 15/015 had warmer air within the 1st 30 days than those of 2nd trial (Table 3). Such warmer climate corresponded with higher RH and lower speed of wind than 2nd trial. However, the period of adoption the moisture levels, of the 1st trial recorded lower air temperatures and RH with higher wind speed than 2nd trial. Such climatic differences between both trials recorded variable thermal units (G.D.D) in both trials and both periods (Table 3).

**Table (1). Significance of mean squares due to moisture levels (M.L.), faba bean cultivars (CVs) and their interaction under first pot experiment during 2014/2015 season.**

| Source of Variation | M.L | CVs. | CVs x M.L |
|---|---|---|---|
| Shoot length (cm) | 60.325** | 49.846** | 10.433* |
| Number of leaves | 21.01** | 9.96** | 1.05* |
| Leaflet area ($cm^2$) | 96.37** | 21.49** | 13.43** |
| Root dry wt ( RDwt),g | 0.731** | 0.413** | 0.146** |
| Shoot Dwt (ShDwt),g | 116.492** | 13.361** | 3.188** |
| R/ ShDwt | 17.239** | 2.702** | 2.514** |
| Plant dry wt(PDwt), g | 135.090** | 16.334** | 3.531** |
| Relative RDwt to PDwt | 9004.774** | 545.508** | 294.760** |
| RWC (%) | 2693.325** | 66.781 ns | 114.939** |
| Leaflet Proline content ($mg\ g^{-1}$) | 81.820** | 1.897** | 0.637** |

ns, * and ** indicate insignificant, significant at 5% and at 1% levels of probability.

**Table (2): Significance of mean squares of combined analysis over both pot trials (E) using the data of 5 common faba bean cultivars (CVs) under 3 moistures levels (M.L).**

| Trait | Sources of variation | | | | | | |
|---|---|---|---|---|---|---|---|
| | E | M.L | M.L x E | CVs | CVsxE | CVsxM.L | CVsxM.LxE |
| Shoot L, cm | 12.21ns | 96.45** | 1.86ns | 100.65** | 3.09ns | 15.23** | 4.18ns |
| Root Dwt, g | 4.05** | 0.58** | 0.35** | 0.32** | 0.45** | 0.11** | 0.19** |
| Shoot Dwt,g | 56.60** | 38.63** | 24.93** | 6.16** | 5.49** | 1.62** | 1.50** |
| R/Sh | 5.76** | 9.69** | 8.75** | 2.59** | 2.09** | 2.23** | 2.25** |
| Plant Dwt, g | 90.94** | 48.61** | 29.65** | 8.36** | 5.63** | 2.11** | 1.42** |
| RRP Dwt | 462.8ns | 3611.3** | 2470.4** | 393.5** | 531.7** | 242.9** | 244.1** |
| RWC | 425.63** | 849.43** | 170.99** | 99.83* | 10.91ns | 91.66** | 32.81ns |

ns, * and ** indicate insignificance, significance at 5% and significance at 1 %, respectively.

**Table (3): Means of climatic parameters during the periods ( 50 days) of first (T1) and second (T2) pot trials in addition to growing degree days (G.D.D) during 2014/2015.**

| Period | Temp.◦ C | | | | RH (%) | | Wind (km/h) | |
|---|---|---|---|---|---|---|---|---|
| | M.T1 | M.T2 | G.D.DT1 | G.D.DT2 | M.T1 | M.T2 | M.T1 | M,T2 |
| 1st 30 days | 17.5 | 15.2 | 470.5 | 410.5 | 60.4 | 45.6 | 6.8 | 9.4 |
| 2nd 20 days | 12.9 | 16.6 | 221.5 | 295.5 | 49.4 | 57.2 | 11.4 | 8.5 |
| General mean | 15.7 | 15.8 | 692.0 | 706.0 | 56.0 | 50.2 | 8.6 | 9.0 |

## Mean Performance and Extent of Variation

Water stress progressively significantly reduced the growth of the faba bean genotypes (Tables 4&5). Under water stress conditions the plant dry weight amounted about 25% of the control plants under both trials. However, it's apparent that root/shoot Dwt (R/S Dwt), as well as the percentage of root Dwtto plant dry weight (RRP Dwt) were significantly increased in medium and stress moisture levels.

Under both environmental conditions, the growth and accumulation of dry matter as well as RWC were significantly reduced as moisture levels decreased. However, the R/Sh, RRP and leaflet proline content were tended in opposite direction (1st trial). In other words, the ratios of accumulated dry matter in roots to those in shoots or whole plant were increased due to the reduction of soil moisture. The decreasing the available soil moisture content resulted in progressive declines in RWC of the leaflets by about 2.5 - 6% in plants grown under M and S conditions, compared to normal one. On the contrary, there were concomitant increases in the proline contents due to water stress.

**Table (4): Mean, ranges, broad sense heritability (BSH) and coefficients of genotypic (GCV%) and phenotypic (PCV%) variations of faba bean genotypes under each M.L for traits at first pot experiment.**

| Trait | M.L | Mean | Range | BSH | GCV% | PCV% |
|---|---|---|---|---|---|---|
| R/ShDwt, g | N | 0.27 b | 0.12-0.48 | 0.257 | 20.3 | 40.0 |
| | M | 0.38 b | 0.14-0.77 | 0.516 | 33.6 | 46.8 |
| | S | 1.53 a | 0.60-5.73 | 0.966 | 102.2 | 104.0 |
| Plant Dwt, g | N | 5.76 a | 2.75-9.23 | 0.797 | 34.2 | 38.3 |
| | M | 3.98 b | 2.37-7.68 | 0.861 | 37.9 | 40.8 |
| | S | 1.53 c | 0.87-2.20 | 0.922 | 34.0 | 35.4 |
| RRP Dwt, g | N | 20.2 c | 11.0-29.1 | 0.464 | 19.9 | 29.1 |
| | M | 25.4 b | 12.1-33.7 | 0.565 | 24.4 | 32.4 |
| | S | 52.5 a | 34.9-85.0 | 0.852 | 29.2 | 31.6 |
| RWC | N | 68.06 a | 61.73-75.69 | 0.767 | 6.6 | 7.5 |
| | M | 61.55 b | 57.21-70.52 | 0.515 | 4.6 | 6.4 |
| | S | 49.39 c | 38.83-55.71 | 0.536 | 11.2 | 15.3 |
| Leaves Proline Content | N | 0.8087 c | 0.3646-1.2313 | 0.985 | 36.8 | 37.0 |
| | M | 1.6644 b | 1.0726-2.7900 | 0.916 | 35.1 | 36.7 |
| | S | 3.9993 a | 3.0454-5.7362 | 0.930 | 18.6 | 19.3 |

Means of the same trait followed by the same letter are not statistically different at 5% level of probability.

**Table (5): Mean, ranges, broad sense heritability (BSH) and coefficients of genotypic (GCV%) and phenotypic (PCV%) variations of 5 faba bean genotypes under each M.L combined over both trials.**

| Trait | M.L | Mean | Range | BSH | GCV% | PCV% |
|---|---|---|---|---|---|---|
| R/ShDwt, g | N | 0.374b | 0.12-0.71 | 0.000 | 0.00 | 45.27 |
| | M | 0.443b | 0.24-0.73 | 0.000 | 0.00 | 43.62 |
| | S | 1.391a | 0.38-5.73 | 0.048 | 23.55 | 107.81 |
| Plant Dwt, g | N | 3.80a | 1.16-9.17 | 0.179 | 19.11 | 45.19 |
| | M | 2.65b | 1.20-4.82 | 0.346 | 18.03 | 30.66 |
| | S | 1.26c | 0.87-2.03 | 0.000 | 0.00 | 30.74 |
| RRP Dwt, g | N | 25.79c | 11.02-40.76 | 0.000 | 0.00 | 12.17 |
| | M | 28.77b | 18.80-41.07 | 0.000 | 0.00 | 12.43 |
| | S | 46.11a | 27.31-85.04 | 0.189 | 11.66 | 26.84 |
| RWC | N | 66.54a | 59.15-72.04 | 0.660 | 6.36 | 7.83 |
| | M | 62.82b | 58.52-67.74 | 0.000 | 0.00 | 4.72 |
| | S | 56.05c | 41.13-66.51 | 0.405 | 7.30 | 11.47 |

Means of moisture levels followed by same letter are not statistically different at 0.05 level of probability.

Judging by ranges of genotypes within each moisture level, water stress broadens the ranges of all traits except plant dry wt during both data manipulations (Tables 4 & 5). The ranges under stress conditions of 10 cvs and 5 ones, were wide by about 10 , 2 and 2 folds for R/S, RRP Dwt and RWC, respectively. However, leaflet proline content recorded in water stress level 3 folds as much as in normal watering regime. These wider ranges of genotypic performances in stress treatment showed higher lower limits in all traits except RWC than those in normal and medium ones. On the other hands, so large higher limit could be observed only for R/Sh and RRPD wt. Plant Dwt ranged narrower under stress than in other non-stressed two treatments (from 8 in N to 4 in M and to about 1 in water stress). This drastic effects of water stress on plant dry wt as least mean performance and narrow range may be due to the adverse effects of drought on dry matter accumulation. This is obviously corresponding to similar negative effects only on shoot dry wt rather than root. The 10's CVs trial recorded higher genotypic and phenotypic variations and BSH under stress than under other non-stressed levels for R/Sh. Plant Dwt under all investigated levels recorded similar GVC% and PVC%, but gradual increase in BSH from N (0.797 to 0.922). For RRP Dwt and proline content, similar genotypic and phenotypic coefficients of variation were recoded under M and S for first trait as well as N and M levels for proline. The broad sense heritability were increase from 0.464 in N to 0.852 in S level for RRP Dwt, while it was more than 0.92 in all treatments for Proline.

The results of combined analyses over both environments, didn't able to detect any genotypic variation among the five cultivars under N and M (for R/Sh and RRP), under S (for Pl Dwt) and under M for RWC. Due to that combined analysis is efficient than single environment trial for extracting the genotypic variation from environmental inflation, BSH in this manner was lower than those estimated from only one trial (Tables 4&5).

**The Rates of Change Due to Reduction of Soil Moisture**

As previously mentioned, variances due to10cultivars and CVs x M.L were highly significant for all studied traits except of CVs for RWC. This revealed that the studied 10 faba bean genotypes performed differently for these traits from watering level to another. To explore and quantify the genotypic responses to moisture reduction, regression analyses was carried out of performances to the AW percentages and the significance within and between genotypes were calculated.

Differences among mean performance of genotypes within normal (N) and stress (S) watering levels were insignificant for R/S and PlDwt (Table 6). Likewise, genotypes for RRP Dwt under normal irrigation exhibited slight difference. This indicated that under water stress the growth and dry matter of shoots were depressed relatively higher than those of roots. The rates of changes (regression coefficients) due to the reduction of available water, common regression coefficients were significantly negative for all tabulated traits except for R/S, RRP and Proline. This means that the R/S, RRP and Proline content of leaves were increased significantly as soil moisture levels decreased .However, the obtained rates of changes of tested genotypes , i.e coefficients of b for all traits were differed significantly as heterogeneity tests indicated. This proved that the tested faba bean genotypes possessed intrinsic variation of responses to water deficit during the early stage of growth. The R/Sh rates of changes of all genotypes were significance except of G.843, C.5and C. 49.Thus only 7 faba bean genotypes seemed possess higher R/Sh ratio , which may help them for uptake the limited soil moisture. In this manner, M.1 cultivar recorded about 10 fold as much as higher (0.11) of other genotypes (0.01or 0.02) of b coefficients. The relative root to plant dry wt (RRP) was positive and significant for all genotypes except C.49. These positive rates of increased RRP were double higher in M.1, S.1 and C.4 than other genotypes. All genotypes possessed significantly the ability of increment leaves proline content as a result of decreasing moisture levels.

The obtained results of the present investigation showed that the moisture levels significantly affected the growth parameters, RWC, and the proline contents of the 10-faba bean genotypes. Moreover, there were remarkable differences among genotypes grown under each moisture regime.

Previous studies on faba bean and other crops reported that drought significantly caused reductions in cell division, cell enlargement and differentiation (Khan *et. al.*,[6]; Siddiqui *et al.*,[7]). As has been reported by Nilsen and Orcutte [17], drought affects the regulations of the chemical processes that take place in the plant, such as the phyto hormones, which consequently act as stimulators or inhibitors of growth. Pospišilová [18] indicated that drought induced the synthesis of abscisic acid in the roots, which transports as a “root signal” to different parts of the plant. Abscisic acid brings out several processes such as stomatal closure, synthesis of compatible osmotica, reduction in transpiration, etc.

The genotype, which is able to maintain growth, dry matter, yield, as well as, hydration during the water stress conditions, would be more drought tolerant and stable than others grown under comparable conditions (Khazaei *et al.*,[19]). According to Grzesiak *et al.*,[20], the greater drought resistance of faba bean cv. Gobo results from more extensive length and greater mass of its roots. Studies have indicated that the high allocation of photosynthates to roots increases the efficiency of water uptake in dry soils (Nielsen et al., [21]) but may be a cost to grain production.

The reductions of the RWC and the increase of the proline contents in the leaflets of the investigated faba bean genotypes subjected to water stress conditions have been widely reported in many wild and cultivated plants (Szabados and Savouré, [22]; Blum, [8]), including faba beans (Khan *et al.*,[6]; Ammar *et al.*,[23]; Siddiqui *et al.*, [7]).Examination of the RWC values under the water stress conditions (S) indicates that the differences among genotypes are either reflects their capacity to absorb their water requirements from the soil and/or to reduce their water loss through transpiration. The net result would be the preservation of the absorbed water and the improvement of hydration of the whole plant. Studies have indicated that the high capability of the plant to maintain water during drought period is a peculiar mechanism that allows plants to tolerate water stress (Blum, [24]). It is apparent that the massive reductions of the RWC of the leaflets of the genotypes grown under water stress indicate that they are sensitive to drought. For example, the genotypes M.1, M.3, C.4 and N.1 appeared to be the most sensitive ones since their lower RWCs under stress conditions, which corresponded to high rates of reductions RWC. In spite of that cultivar C.5shared C.4 in highest rate of RWC reduction (b=-0.72**), the first maintained significantly higher RWC (=55.71%). This may be due to the highest rate and content of increase proline (b=0.010** & 5.74 mg $g^{-1}$ fresh wt) of C.5, which is two folds as much as higher of C.4 (b=0.05**& 3.83 mg $g^{-1}$ fresh wt). Cultivars exhibited lower declines of RWCs due to AW reductions corresponded to higher RWC under moisture stress may be considered as tolerant to water stress (i.e G.843 and C. 49).

It has been well reported that when the plants grow under environmental stresses such as drought and salinity, they accumulate solutes like sugars, amino acids and amines in their cells (Blum, [8]). The accumulation of solutes is known as osmotic adjustment, which maintains cellular turgor pressure through the osmotic influx of water and protects the structure and function of cellular components (Gomes *et al.*, [25]). Proline amino acid has been associated with drought stress (Hayat *et al.*, [26]). It accumulates in the cytoplasm without affecting the enzymes and the cellular structures (Szabados and Savouré, [22]). The increase in the proline contents in the leaflets of the investigated faba bean genotypes grown under conditions of medium (M) and stress (S) regimes is a phenomenon reported in many previous studies. Proline accumulation in leaflets of faba bean genotypes obtained from different geographical origins and grown under the extreme level of water stress have been documented by Siddiqui *et al.* [7]. They indicated that the level of proline depends upon the magnitude of water stress. Abdelgawad *et al.* [27] reported that both salinity and drought stress resulted in significant increases in the contents of acyclic and cyclic amino acids in the leaves of faba bean. Moreover, they found that proline reached the highest values in genotypes under water and salinity stress conditions.

In order to identify the contribution of proline to the osmotic potential of the cells of the investigated faba bean genotypes, we selected the genotype C.5, which showed the high proline contents under normal (N) and water stress (S) conditions. The concentrations of proline were expressed on tissue water content bases to calculate their contributions to the osmotic potential. The calculated osmotic potential due to proline ranged from -0.021 to -0.118 MPa in control and water stressed plants, respectively. Since we did not measure the osmotic potential of the tissues, we used the published data of Khan et al. [13], who reported that the minimum osmotic potential of field grown faba beans ranged between -0.79 to -1.00 MPa under conditions of normal irrigation and water stress, respectively. Our calculations revealed that the levels of proline of C.5 grown under medium (M) and water stress (S) conditions contributed 5.3% and 12.8% of the total osmotic potential (which is equal to -1.00 MPa, according to the data taken from Khan *et al.* [13]. The percentages contribution of proline to the total predicted osmotic potential of the genotype C.5 indicates that this amino acid might play a role in the overall osmotic adjustment of the cells. Our calculations, which were based on the actual concentrations of proline in tissues, are not in agreement with previous studies and reports on faba beans (Amede *et al.*, [11]; Katerji *et al.*, [28]; Khan *et al.*, [6]) who pointed that there is no evidence of osmotic adjustment in faba beans. They also indicated that the solute accumulation in the cells results from severe water loss under drought conditions.

**Table (6). Performance of investigated faba bean genotypes under normal (N)and stressed (S) watering levels for some growth parameters, relative water contents, and proline contents and the regression coefficients (b) of performancetomoisture levels in first pot trial during 2014/2015.**

| Parameters | Moisture level | Genotypes | | | | | | | | | | $LSD_{0.05}$ |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | M 1 | M 3 | S 1 | G 843 | C 4 | C 5 | C 25 | C 49 | N 1 | LuzDE | |
| Root Dwt (R) | N | 1.78 | 0.77 | 1.07 | 0.90 | 0.53 | 0.88 | 0.97 | 1.10 | 1.62 | 1.00 | 0.35 |
| | S | 1.05 | 0.78 | 1.13 | 0.84 | 0.58 | 0.68 | 0.47 | 0.40 | 0.91 | 0.68 | 0.35 |
| | b | -0.02* | 0.00ns | 0.00ns | -0.00ns | 0.00ns | -0.01ns | -0.01** | -0.01** | -0.01* | -0.01ns | -0.01**[1)] |
| Shoot Dwt (Sh) | N | 5.53 | 5.48 | 3.42 | 3.75 | 2.22 | 4.63 | 3.18 | 2.97 | 7.62 | 8.17 | 1.81 |
| | S | 0.18 | 0.92 | 0.41 | 1.44 | 0.33 | 0.98 | 0.42 | 0.47 | 1.30 | 1.35 | 1.81 |
| | b | -0.11** | -0.09** | -0.06** | -0.05* | -0.04** | -0.07** | -0.06* | -0.05** | -0.13** | -0.14** | -0.08**[1)] |
| R/Sh | N | 0.35 | 0.14 | 0.32 | 0.26 | 0.25 | 0.20 | 0.36 | 0.48 | 0.22 | 0.12 | ns |
| | S | 5.73 | 0.91 | 2.78 | 0.60 | 1.89 | 0.86 | 1.15 | 1.08 | 0.73 | 0.60 | 0.54 |
| | b | 0.11** | 0.02** | 0.05** | 0.01ns | 0.03** | 0.01ns | 0.02** | 0.01ns | 0.01* | 0.01* | **0.03****[1)] |
| PlDwt, g | N | 7.32 | 6.25 | 4.48 | 4.65 | 2.75 | 5.52 | 4.15 | 4.07 | 9.23 | 9.17 | 1.92 |
| | S | 1.23 | 1.70 | 1.55 | 2.28 | 0.92 | 1.67 | 0.88 | 0.87 | 2.20 | 2.03 | ns |
| | b | -0.12** | -0.09** | -0.06** | -0.05** | -0.04** | -0.08** | -0.07** | -0.06** | -0.14** | -0.14** | **-0.09****[1)] |
| RRP, Dwt,g | N | 25.4 | 12.20 | 24.10 | 20.50 | 19.80 | 16.70 | 25.40 | 29.10 | 18.20 | 11.00 | 15.4 |
| | S | 85.00 | 46.30 | 73.10 | 37.40 | 64.60 | 40.90 | 53.20 | 48.10 | 41.40 | 34.90 | 15.4 |
| | b | 1.19** | 0.68** | 0.98** | 0.34* | 0.90** | 0.49* | 0.55* | 0.38ns | 0.46* | 0.48* | **0.65****[1)] |
| RWC (%) | N | 63.71 | 75.69 | 70.69 | 61.73 | 74.61 | 69.34 | 72.04 | 64.48 | 65.84 | 62.48 | 10.36 |
| | S | 41.13 | 48.02 | 54.50 | 55.43 | 38.86 | 55.71 | 54.16 | 59.19 | 38.83 | 48.09 | 10.36 |
| | b | -0.45** | -0.55** | -0.32** | -0.13ns | -0.72** | -0.72** | -0.36* | -0.11ns | -0.54* | -0.29** | **-0.37****[1)] |
| Proline content (mg $g^{-1}$ fresh wt) | N | 0.61 | 1.02 | 0.37 | 1.14 | 1.21 | 0.96 | 0.68 | 1.00 | 0.44 | 0.64 | 0.44 |
| | S | 3.64 | 4.46 | 3.19 | 4.33 | 3.83 | 5.74 | 3.53 | 4.25 | 3.05 | 3.99 | 0.44 |
| | b | 0.06** | 0.07** | 0.06** | 0.06** | 0.05** | 0.10** | 0.06** | 0.07** | 0.05** | 0.07** | **0.06***[1)] |

ns, * and ** indicate insignificance, significance at 5% and significance at 1 %, respectively.

1) Common regression coefficient of genotypes in the same trait corresponded by the significance of testing the heterogeneity of b's.

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