# GENETIC POTENTIAL AND ASSOCIATION AMONG MORPHO-PHYSIOLOGICAL TRAITS OF *PETUNIA* INBRED LINES

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**Abstract.** *Petunia hybrida* is considered as an important and beautiful ornamental flowering plant, grown throughout the world for its beauty and attractiveness. Various petunia hybrids have been produced by different countries. The present study was carried out to investigate the higher seed yield petunia line developed through selfing to produce $F_1$ hybrids during the experimental seasons of the 2011-2014 period. The lines IAGS-P8, IAGS-P9 and IAGS-P11 performed well, both in the cluster analysis and minimum spanning tree, the inbred lines IAGS-P8 and IAGS-P9 showed to a higher similarity percentage. From statistical and regression analysis showed that stomata conductance, chlorophyll ‘a’ contents, chlorophyll ‘b’ contents, flowers per plant, flower fresh weight, seed area, seed weight and abscisic acid contributed higher to seed yield per plant in petunia. The higher abscisic acid concentration suggested that the petunia genotypes may be used to develop better seed yields and higher number of flowers per plant in petunia under environmental stress. This method proved to be more efficient as it reduced the cost, time and efficacy for better selection in petunia improvement program. Still, further studies are required which should cover different years and locations.

**Keywords:** *Petunia hybrida, abscisic acid, multivariate analysis, heritability, genetic advance*

## Introduction

*Petunia hybrida* is an important and the mostly used ornamental plant throughout the world from several years as commercially grown ornamental plant. *Petunia hybrida* is an important and mostly used genera to develop *Petunia* new varieties and hybrids. Griesbach (2007) has reported that *Petunia* has an old history in sense of development as an ornamental plant as compared with new emerging ornamental crops plant species. Ganga et al. (2011) suggested that the production of hybrid seed has been adapted by various ornamental crop plants breeding program to develop new colour and varieties of ornamental plants. It has been found that there are 20 South American origin species of

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*Petunia* (Selaru, 2008), 14 herbaceous species (Toma, 2009). Selaru (2008) reported that the specie *Petunia hybrida* Hort. (*P. axillaris Lam.* × *P. violacea* Lindl.) has been presented with higher decorative value. It has been confirmed from findings of various researchers that the 14 familiar species are consisted of genus *Petunia* (Stehmann et al., 2009). The *Petunia hybrida* Hort. as an ornamental plant species, used as ornamental plant in loans, fields and gardens for its abundant flowering ability (Toma et al., 2011). Dole and Wilkins (1999) reported that most of the *Petunia* species have been developed in Brazil, Uruguay or Argentina. In modern era a large number of cultivars have been developed in the form of varieties, hybrids and cultivars with variety of colors and patterns. Depending on flower size, color and habit, four groups of *Petunias* have been defined as, multiflora, milliflora, grandiflora, ground cover or spreading. Even every year new varieties of *Petunia* have been developed by ornamental breeders (Toma, 2009).

Bender (2006) found that the new and modern species of petunias are hybrids in nature like garden petunia (*Petunia axillaris*) or ambrosial white and violet *petunia* (*P. integrifolia*, and *P. violacea*). The old or wild type *petunia* species, varieties or hybrids use has been less down now a day. *Petunia* is grown throughout the world for its beauty, higher growth rate, different color ranges and continued flowering up to about 6 months of plant life cycle. It blossoms most abundantly under hot summers conditions (Bala, 2012; Selaru, 2008). Erwin (2006) found that the higher blooming ability, *petunia* is even grown in baskets, landscaping by the forms, in alloyed trays on roads and even in room open spaces (Erwin, 2006). The present study was conducted to develop inbred lines of petunia through selfing at growing seasons. The data of various morphological, physiological and seed yield traits was recorded to access the performance of inbred lines under development. The identification of promising inbred lines was also done.

## Material and methods

The prescribed study was carried out in the research area of Institute of Agricultural Sciences, University of the Punjab Lahore, Pakistan located at 31.4779° N, 74.2623° E with average higher temperature of about 37 °C with highest up to 45 °C during summer season, 11 °C with lowest 3 °C during winter season. Twelve petunia lines, IAGS-P1, IAGS-P2, IAGS-P3, IAGS-P4, IAGS-P5, IAGS-P6, IAGS-P7, IAGS-P8, IAGS-P9, IAGS-P10, IAGS-P11 and IAGS-P12 were selected and grown in the field during 2014, selfing of all the lines was carried out for three successive growing seasons (2011-13) to develop inbred lines, the selfed seed were collected to develop next generation (*Figs. 1* and *2*). The selfed seed was grown in three replication under completely randomized block design during growing season of 2014, and data for various traits of 10 selected plants from each of three replications was recorded during all three growing seasons and average of data was used for results and interpretation, the traits were included photosynthetic rate (A), leaf temperature (LT), transpiration rate (E), stomata conductance (gs) and Sub-stomata $CO_2$ Concentration (Ci) through the use of IRGA (Infrared Gas Analyzer, Model: LI-6400XT), chlorophyll ‘a’ content (Chl. a), chlorophyll ‘b’ content (Chl. b) in fresh matter was measured using the dimethyl sulfoxide extraction method (Hiscox and Israelstam, 1978), leaves per plant (LPP), plant height (PH), stem diameter (SD), flowers per plant (FPP), leaf length (LL), leaf width (LW), leaf area (LA), fresh leaf weight (FLW), fresh stem weight (FSW), flower

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weight (FW), seeds per fruit (SPF), 100-seed weight (HSW), seed area (SA), capsule weight (CW), abscisic acid (ABA) by using HPLC method (Seo and Koshiba, 2002), and seed yield per plant (SYP) were collected. Water use efficiency (WUE) was recorded through using given formula:

$$\text{Water use efficiency}\left(\%\right) = \frac{\text{Transpiration rate}}{\text{Photosynthetic rate}} \times 100$$

The collected data was statistically analyzed by using analysis of variance technique, principal component analysis, correlation analysis, regression analysis, factor analysis, spinning tree and cluster analysis (Steel et al., 1997) through using GenStat 18 (SP1) version.

***Figure 1.*** *Twelve lines petunia hybrida grown in filed*

### *Heritability estimate*

Broad sense heritability was calculated by using genotypic, phenotypic and environmental variances according to Burton (1951):

$$\text{Broad sense heritability} = \frac{\left(\partial^2{}_g + \partial^2{}_e\right) - \partial^2{}_e}{\partial^2{}_g + \partial^2{}_e} \times 100$$

$\partial^2_g$ = genotypic variance, $\partial^2_e$ = environmental variance, $\partial^2_g + \partial^2_e$ = phenotypic variance, $(\partial^2_g + \partial^2_e) - \partial^2_e$ = genotypic variance.

Genetic advance (GA) was calculated by the following formula (Falconer, 1989):

$$\text{GA} = \sigma_p x\ h^2 x\ i$$

where: $\sigma_p$ = the phenotypic standard deviation; $h^2$ = estimate of broad sense heritability; i = constant value (1.755) that reflects selection intensity (10%).

***Figure 2.*** *Close view of different petunia hybrid in field and pot (for transplanting in field)*

## Results and discussions

It was persuaded from the results (*Table 1*) that significant differences were found for all studied traits. Broad sense heritability was found higher for photosynthetic rate (90.825%), stomata conductance (80.268%), sub-stomata $CO_2$ concentration (96.035%), water use efficiency (89.847%), leaves per plant (98.750%), plant height (92.613%), leaf length (83.584%), flowers per plant (92.911%), leaf area (84.460%), fresh stem weight (85.266%), seeds per fruit (96.379%) and abscisic acid (85.205%) while lower for chlorophyll b (23.527%), leaf width (37.084%), fresh leaf weight (24.074%), flower weight (26.145%), seed area (26.781%) and seed yield per plant (4.359%). Genetic advance was found higher for all of the studied traits seeds per fruit. Higher heritability suggested that the selection of petunia lines on the basis of these traits for the development of hybrids may be fruitful. Various researchers while working on different crop plants have described about higher heritability for these traits as reported in our study (Ali et al., 2013, 2014; Fawad et al., 2017). Higher genetic advance suggested that the selection of lines may also be helpful to develop synthetic varieties. The similar findings for different crops have been reported by various researchers (Ali et al., 2013, 2016). It was found from results (see *Appendix*) that the line IAGS-P8, IAGS-P9 and IAGS-P11 performed better than all other lines. Correlation analysis provides an opportunity to the research to select genotypes of crop plant to improve crop plant growth and production. In our study, significant correlation (*Table 2*) was found for photosynthetic rate with chlorophyll ‘a’ contents, sub-stomata $CO_2$ concentration, water use efficiency, plant height, flowers per plant, leaf width and abscisic acid. Abscisic acid was found to be significantly correlated with most of the studied traits including seed yield per plant. Stomata conductance showed also significant correlation with transpiration rate, water use efficiency, flowers per plant, leaf area, fresh shoot weight, seeds per fruit, abscisic acid and leaf width. Seed yield per plant was significantly correlated with photosynthetic rate, leaf temperature, leaves per plant, stem diameter, seed weight and abscisic acid. The positive and significant correlation suggested that the selection of lines to develop hybrids and synthetic varieties may be helpful to improve the growth and development of petunia, the significant correlation of abscisic acid with morphological, seed yield and physiological traits indicated that the selection of petunia lines on the basis of good abscisic acid production may be fruitful to improve drought tolerance in petunia (Mahmood et al., 2017; Sah et al., 2016; Skubacz et al., 2016; Zao et al., 2016; Saradadevi et al., 2017). Kaczperski et al. (1991) estimated growth of the petunia and development in combined effects of light and temperatures. The adverse effect of an optimum temperature environment for minimum time to *petunia hybrida* was begin to be 25 °C, and a minimum circadian light intensity for adequate growth was begin to be 13 $molm^{-2}$ $d^{-1}$. The adeptness for petunia to growth and development with increase in light and temperature has afresh been appeared, so there is befalling to optimize development, crop plant growth, crop scheduling and higher with ecology changes in light and increase in temperature. However, the accessible delay of growth and development with top abundance and the access of $CO_2$ in an optimized environment showed not extensively have adverse effects (Ahmad and Tahir, 2017; Blanchard and Runkle, 2009; Mustafa et al., 2017).

The stepwise regression (*Table 3*) was performed to find out the traits that were highly contributing to the seed yield per plant of petunia, from results it was predicted that stomata conductance (120.311), chlorophyll ‘a’ contents (2.775), chlorophyll ‘b’ contents (2.1040, flowers per plant (2.484), flower fresh weight (11.364), seed area (7.062), seed weight (31.497) and abscisic acid (15.356) contributed higher to seed yield per plant in petunia but it could be biased as previous literature also reported the error effect of stepwise regression (El-Badaway and Mehasen, 2011; Ahmad et al.; 2012, 2016) while handling a large number of independent variables. The Intercept = 128.387, $R^2 = 0.832$, Adjust $R^2 = 0.276$ and Standard Error = 0.728 was found with expected regression equation as follows:

$$\begin{aligned} Y = {} & 128.387 + (0.224X_1) + (-0.235\,X_2) + (2.775\,X_3) + (2.2.104X_4) + (120.311X_5) \\ & + (-4.418X_6) + (-0.007X_7) + (-0.070X_8) + (-0.252X_9) + (0.116X_{10}) + (0.004X_{11}) \\ & + (2.484X_{12}) + (-0.115X_{13}) + (-17.234X_{14}) + (-80.073X_{15}) + (11.364X_{16}) + (-10.121X_{17}) \\ & + (0.068X_{18}) + (-12.101X_{19}) + (-0.033X_{20}) + (7.062X_{21}) + (31.497X_{22}) + (15.356X_{23}) \end{aligned}$$

Various researchers have used PCA (Principal Component Analysis) to evaluate the effects of independent traits (*Table 4*) in large number for selecting the fruitful variability percentage from a large population (Fawad et al., 2017; Ali et al., 2014). It has been reported from study conducted by Greenacre (2010) that the eigenvalues (used in PCA) shows the primary importance of numerical diagnostics for assessing the variation which a number of large traits/variables contributed for the dependent structure also to display the data matrix in a graphical form.

***Table 1.*** *Genetic components for various morpho-physiology and yield traits of petunia*

| Traits | M.S | G.M ± SE | GV | GCV % | PV | PCV % | EV | ECV % | $h^2$bs% | GA% |
|---|---|---|---|---|---|---|---|---|---|---|
| Photosynthetic rate ($\mu gCO_2 s^{-1}$) | 124.356* | 15.114 ± 0.0094 | 40.102 | 168.621 | 44.153 | 176.933 | 4.051 | 53.593 | 90.825 | 75.097 |
| Leaf temperature (°C) | 139.563* | 22.184 ± 0.0613 | 38.703 | 135.136 | 62.157 | 171.255 | 23.454 | 105.198 | 62.267 | 40.651 |
| Chlorphyll a (mg $g^{-1}$ fr. wt.) | 13.467* | 3.219 ± 0.0579 | 4.074 | 112.848 | 5.319 | 128.943 | 1.245 | 62.383 | 76.593 | 96.905 |
| Chlorphyll b (mg $g^{-1}$ fr. wt.) | 17.972* | 1.268 ± 0.0386 | 2.875 | 135.008 | 12.221 | 278.339 | 9.346 | 243.404 | 23.527 | 91.503 |
| Stomata conductance (mmol $m^{-2}$ $s^{-1}$) | 1.294* | 0.042 ± 0.0068 | 0.399 | 361.210 | 0.497 | 403.169 | 0.098 | 179.089 | 80.268 | 324.911 |
| Transpiration rate (mm $day^{-1}$) | 1.029* | 0.914 ± 0.0176 | 0.272 | 55.482 | 0.485 | 74.087 | 0.213 | 49.097 | 56.082 | 77.574 |
| Sub-stomata $CO_2$ concentration (µmol $mol^{-1}$ $CO_2$) | 238.323* | 149.119 ± 0.8165 | 78.363 | 72.548 | 81.598 | 74.030 | 3.235 | 14.740 | 96.035 | 10.226 |
| Water use efficiency (%) | 37.356* | 7.781 ± 0.1471 | 12.000 | 132.920 | 13.356 | 140.229 | 1.356 | 44.682 | 89.847 | 84.844 |
| Leaves per plant | 234.974* | 87.742 ± 0.4714 | 77.996 | 94.820 | 78.983 | 95.418 | 0.987 | 10.669 | 98.750 | 17.755 |
| Plant height (cm) | 209.803* | 53.719 ± 0.0910 | 68.123 | 110.474 | 73.557 | 114.795 | 5.434 | 31.201 | 92.613 | 24.974 |
| Stem diameter (cm) | 1.073* | 0.532 ± 0.0012 | 0.311 | 78.057 | 0.450 | 93.879 | 0.139 | 52.157 | 69.134 | 159.345 |
| Flowers per plant | 398.244* | 147.16 ± 0.4714 | 129.456 | 95.819 | 139.333 | 99.407 | 9.877 | 26.467 | 92.911 | 13.651 |
| Leaf length (cm) | 36.357* | 7.121 ± 0.0216 | 11.374 | 134.112 | 13.608 | 146.692 | 2.234 | 59.435 | 83.584 | 85.567 |
| Leaf width (cm) | 5.459* | 1.618 ± 0.0294 | 1.162 | 92.533 | 3.134 | 151.951 | 1.972 | 120.527 | 37.084 | 84.878 |
| Leaf Area ($cm^2$) | 39.023* | 6.124 ± 0.1392 | 12.256 | 138.894 | 14.511 | 151.133 | 2.255 | 59.578 | 84.460 | 88.879 |
| Fresh leaf weight (g) | 3.214* | 0.661 ± 0.0249 | 0.522 | 89.340 | 2.169 | 182.086 | 1.647 | 158.662 | 24.074 | 95.103 |
| Fresh stem weight (g) | 204.224* | 46.321 ± 0.0535 | 64.367 | 114.352 | 75.490 | 123.838 | 11.123 | 47.536 | 85.266 | 26.413 |
| Flower weight (g) | 2.029* | 0.636 ± 0.0205 | 0.348 | 76.110 | 1.332 | 144.736 | 0.984 | 124.385 | 26.145 | 98.045 |
| Seeds per fruit | 998.267* | 869.169 ± 0.4714 | 328.640 | 61.597 | 340.986 | 62.743 | 12.346 | 11.939 | 96.379 | 3.606 |
| 100-seed weight (mg) | 65.324* | 13.131 ± 0.0082 | 17.363 | 120.043 | 30.597 | 159.354 | 13.234 | 104.801 | 56.748 | 45.721 |
| Seed area (mm) | 2.091* | 0.418 ± 0.0052 | 0.365 | 101.044 | 1.362 | 195.254 | 0.997 | 167.075 | 26.781 | 153.556 |
| Seed yield per plant | 1.129* | 52.213 ± 0.0294 | 0.099 | 4.354 | 1.092 | 14.462 | 0.993 | 13.791 | 9.066 | 5.769 |
| Capsule weight (mg) | 98.672* | 115.121 ± 0.0618 | 25.447 | 47.015 | 47.779 | 64.423 | 22.332 | 44.044 | 53.259 | 62.284 |
| Abscisic acid (mg/100 g fresh leaf weigh) | 572.897* | 127.126 ± 0.0163 | 180.517 | 125.221 | 211.862 | 135.658 | 31.345 | 52.180 | 85.205 | 18.906 |

*Significant at 5% probability level. Mean Sum of Squares (M.S), Grand mean (G.M), Genotypic variance (GV), Genotypic coefficient of variance (GCV %), Phenotypic variance (PV), Phenotypic coefficient of variance (PCV %), Environmental Variance (EV), Environmental coefficient of variance (ECV %), Broad sense heritability (h2bs %), Genetic advance (GA)

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***Table 2.*** *Correlation among various morpho-physiology and yield traits of petunia*

| **Traits** | **A** | **LT** | **Chl. a** | **Chl. b** | **gs** | **E** | **Ci** | **WUE** | LPP | PH | SD | FPP | LL | LW | LA | FLW | FSW | FW | SPF | HSW | SA | SW | ABA |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| LT | -0.1013 | | | | | | | | | | | | | | | | | | | | | | |
| Chl. a | 0.8128* | -0.0851 | | | | | | | | | | | | | | | | | | | | | |
| Chl. b | -0.0597 | -0.2476 | -0.0181 | | | | | | | | | | | | | | | | | | | | |
| gs | -0.2867 | 0.1704 | -0.1030 | 0.4668* | | | | | | | | | | | | | | | | | | | |
| E | -0.0079 | 0.4530* | -0.0727 | 0.8146* | 0.4579* | | | | | | | | | | | | | | | | | | |
| Ci | 0.3950* | -0.2423 | 0.3196* | 0.0541 | 0.3187 | 0.0697 | | | | | | | | | | | | | | | | | |
| WUE | 0.5112* | -0.2758 | 0.3659* | 0.7206* | 0.4996* | 0.8432* | 0.2287 | | | | | | | | | | | | | | | | |
| LPP | 0.2184 | 0.1949 | -0.1063 | -0.2864 | -0.1038 | -0.6049* | -0.2114 | -0.6657* | | | | | | | | | | | | | | | |
| PH | 0.4889* | 0.0884 | 0.5840* | -0.3759* | -0.2407 | -0.3948* | 0.5473* | -0.0364 | -0.2126 | | | | | | | | | | | | | | |
| SD | -0.0771 | 0.2057 | 0.2103 | -0.1540 | -0.1632 | -0.4087* | 0.1115 | -0.2716 | 0.0482 | 0.5810* | | | | | | | | | | | | | |
| FPP | 0.5809* | -0.3171 | 0.4399* | -0.3461* | 0.6668* | -0.2286 | -0.1656 | -0.4426* | -0.1203 | 0.1310 | 0.1846 | | | | | | | | | | | | |
| LL | 0.0572 | 0.5487* | -0.0572 | 0.3546* | 0.3167 | 0.5373* | -0.0113 | 0.3584* | 0.1298 | 0.6172* | 0.7836* | -0.3034 | | | | | | | | | | | |
| LW | 0.4323* | -0.1097 | 0.2583 | 0.1649 | 0.4055* | 0.2566 | 0.5872* | 0.3416* | -0.3639* | 0.4110* | 0.1859 | -0.3387 | -0.0998 | | | | | | | | | | |
| LA | -0.0988 | 0.5709* | 0.0384 | 0.4181* | 0.4586* | 0.6219* | 0.2168 | 0.4765* | -0.0118 | 0.4380* | 0.6828* | -0.4192* | 0.9250* | 0.2848 | | | | | | | | | |
| FLW | -0.1776 | 0.0755 | 0.2038 | 0.4141* | 0.1044 | 0.0602 | 0.1902 | 0.1690 | 0.3791 | -0.0838 | -0.2435 | -0.3787* | 0.2378 | -0.1561 | 0.1826 | | | | | | | | |
| FSW | 0.2286 | 0.0874 | -0.0975 | 0.8117* | 0.7284* | 0.7186* | -0.1976 | 0.6657* | 0.1219 | 0.4128* | 0.3056 | 0.7602* | 0.5195* | 0.3838* | 0.6544* | -0.3962* | | | | | | | |
| FW | -0.3168 | 0.3892 | 0.2256 | 0.5522* | -0.2800 | 0.5444* | -0.3062 | -0.3209 | 0.1814 | 0.3695* | 0.2475 | -0.0049 | -0.4342* | 0.2129 | -0.3496* | -0.2250 | 0.4368* | | | | | | |
| SPF | 0.3357* | -0.2624 | -0.2190 | 0.0261 | 0.6072* | 0.2327 | -0.3290 | 0.0701 | -0.5112* | -0.0825 | -0.2852 | 0.6372* | -0.0504 | -0.2489 | -0.1388 | -0.2299 | 0.3457* | -0.0509 | | | | | |
| HSW | -0.2110 | -0.0802 | 0.0189 | 0.2805 | 0.2698 | 0.2135 | -0.2069 | 0.1428 | 0.1302 | 0.4799* | -0.2091 | -0.4771* | 0.3224 | 0.3372 | 0.4300* | -0.0049 | -0.5089* | 0.2117 | -0.1950 | | | | |
| SA | -0.2123 | -0.0176 | 0.4387* | -0.3649* | -0.3544* | -0.3596* | -0.2203 | -0.1311 | 0.0090 | 0.4723* | 0.5642* | 0.2387 | -0.3503* | -0.1822 | -0.4146* | -0.4584* | 0.5161* | 0.3473* | -0.0084 | -0.3245 | | | |
| SW | -0.1780 | 0.5446* | 0.1105 | 0.1083 | 0.2293* | -0.0731 | -0.0224 | 0.1543 | -0.1874 | 0.1312 | 0.3928* | -0.1639 | -0.4553* | -0.3195 | -0.5608* | 0.0272 | -0.0047 | -0.2391 | -0.2006 | -0.3778* | 0.2548 | | |
| ABA | 0.4873* | 0.5678* | 0.4295* | 0.6059* | 0.6105* | 0.5155* | 0.1939 | 0.6303* | 0.6055* | 0.0500 | -0.1248 | -0.1071 | 0.2178 | 0.4029* | 0.3521* | -0.1509 | 0.3672* | -0.0245 | 0.4402* | 0.4521* | -0.0221 | 0.5046* | |
| SYP | 0.4686* | 0.3733* | -0.2753 | -0.2215 | 0.3062 | -0.3579* | -0.1311 | -0.3978* | 0.3493* | -0.0664 | 0.3580* | -0.0343 | -0.1842 | -0.1173 | -0.2242 | -0.4212* | 0.1257 | 0.0152 | -0.4284* | -0.0631 | 0.3865* | 0.3295* | 0.5632* |

*Significant at 5% probability level. A = Photosynthetic rate, LT = Leaf temperature, Chl. a = Chlorophyll ‘a’ content, Chl. b = Chlorophyll ‘b’ content, E = transpiration rate, gs = stomata conductance, Ci = Sub-stomata $CO_2$ Concentration, WUE = water use efficiency, LPP = leaves per plant, PH = Plant height, SD = Stem diameter, FPP = Flowers per plant, LL = Leaf length, LW = Leaf width, LA = Leaf area, FLW = Fresh leaf weight, FSW = Fresh stem weight, FW = Flower weight, SPF = Seeds per fruit, HSW = 100-seed weight, SA = Seed area, CW = Capsule weight, ABA = Abscisic acid, SYP = Seed yield per plant

***Table 3.*** *Stepwise regression analysis for various traits of petunia for seed yield*

| | Traits | *Coefficients B* | *Standard error* | *t Stat* | *Cumulative $R^2$* | *Partial $R^2$ %* |
|---|---|---|---|---|---|---|
| $X_1$ | Photosynthetic rate | 0.224 | 0.213 | 1.053 | 0.403 | 40.30 |
| $X_2$ | Leaf temperature | -0.235 | 0.176 | -1.330 | 0.315 | 31.50 |
| $X_3$ | Chlorphyll a | 2.775 | 2.329 | 1.192 | 0.356 | 35.60 |
| $X_4$ | Chlorphyll b | 2.104 | 1.920 | 1.096 | 0.387 | 38.70 |
| $X_5$ | Stomata conductance | 120.311 | 47.385 | 2.539 | -0.126 | 12.60 |
| $X_6$ | Transpiration rate | -4.418 | 3.471 | -1.273 | 0.331 | 33.10 |
| $X_7$ | Sub-stomata $CO_2$ concentration | -0.007 | 0.004 | -1.689 | -0.233 | 23.30 |
| $X_8$ | Water use efficiency | -0.070 | 0.364 | -0.191 | 0.866 | 86.60 |
| $X_9$ | Leaves per plant | -0.252 | 0.157 | -1.607 | 0.249 | 24.90 |
| $X_{10}$ | Plant height | 0.116 | 0.088 | 1.325 | 0.277 | 27.70 |
| $X_{11}$ | Stem diameter | 0.004 | 0.0627 | 0.068 | 0.950 | 95.00 |
| $X_{12}$ | Flowers per plant | 2.484 | 8.886 | 0.279 | 0.798 | 79.80 |
| $X_{13}$ | Leaf length | -0.115 | 0.096 | -1.203 | -0.315 | 31.50 |
| $X_{14}$ | Leaf width | -17.234 | 17.825 | -0.967 | 0.405 | 40.50 |
| $X_{15}$ | Leaf area | -80.073 | 80.280 | -0.997 | -0.392 | 39.20 |
| $X_{16}$ | Fresh leaf weight | 11.364 | 11.769 | 0.966 | 0.406 | 40.60 |
| $X_{17}$ | Fresh stem weight | -10.121 | 5.151 | -1.965 | -0.144 | 14.40 |
| $X_{18}$ | Flower weight | 0.068 | 0.1356 | 0.501 | 0.643 | 64.30 |
| $X_{19}$ | Seeds per fruit | -12.101 | 10.544 | -1.148 | -0.315 | 31.50 |
| $X_{20}$ | 100-seed weight | -0.033 | 0.0589 | -0.563 | 0.604 | 60.40 |
| $X_{21}$ | Seed area | 7.062 | 4.326 | 1.632 | -0.178 | 17.80 |
| $X_{22}$ | Capsule weight | 31.497 | 18.527 | 1.701 | 0.164 | 16.40 |
| $X_{23}$ | Abscisic acid | 15.356 | 30.458 | 0.504 | 0.641 | 64.10 |

Intercept = 128.387, $R^2$ = 0.832, Adjust $R^2$ = 0.276, Standard Error = 0.728

We performed principle component analysis to investigate the traits contributing higher towards seed yield per plant in petunia, four PCA (*Table 4*) were estimated which diverse variation among the studied traits. The PC1, PC2, PC3 and PC4 contributed variations of 29.60%, 16.60%, 13.90% and 10.5% while their cumulative proportion was 29.6%, 46.20%, 60.10% and 70.60% respectively. PC1 and PC2 contributed higher variation for respective studied traits (*Fig. 3a*) the eigenvalues of PCs was higher than 1 (*Fig. 3b*). Through the use of principle component analysis, principle factor analysis was performed to check the traits that were directly and highly associated with the seed yield per plant in petunia. The highly contributing factor traits from factor 1 which contributes 36.20% in total variation were chlorophyll a, chlorophyll b, stomata conductance, transpiration rate, water use efficiency, leaves per plant, stem diameter, leaf length, leaf area, seed yield per plant and abscisic acid (*Table 5*). Fawad et al. (2017) and Ali et al. (2014) suggested that the selection of genotypes on the basis of factor analysis (traits from factor 1) may be helpful to develop higher yield synthetic varieties and hybrids. The traits in factor 2 (from factor loading *Table 5*) showed that the selection on the basis of these traits will not be useful as segregation takes place in the next generation. The higher performance of petunia genotypes for chlorophyll a, chlorophyll b, stomata conductance, transpiration rate, water use efficiency, leaves per plant, stem diameter, leaf length, leaf area, seed yield per plant and abscisic acid indicated that the accumulation of organic compounds will be higher in the plant body. The accumulation of biomass in plant body is essential for the proper and enhanced growth and development of petunia (Huang, 2007; Huang and Yeh, 2009; Ali et al., 2011; Munir et al., 2016), the accumulation is generally in the leaf, stem and flowering parts of the plant body. Our findings are well supported by Filipovic et al. (2014) who demonstrated the role of factor analysis for effective selection criteria in maize breeding program. For understanding of the association among the petunia lines, we performed cluster analysis (Mahmood et al., 2016; Khorasani et al., 2011; Mostafavi et al., 2011; Ali et al. 2015, 2016). The results from clustering showed that the petunia lines IAGS-P8 and IAGS-P9 were highly associated with each other as compared with all other petunia lines (*Fig. 4a*) the association was verified through the development of minimum spanning tree (*Fig. 4b*) that showed very less distance between petunia lines IAGS-P8 and IAGS-P9 through the use of eigen values. The results showed that the petunia lines IAGS-P8 and IAGS-P9 may be used as two separate male or female lines to develop petunia hybrids. The petunia line IAGS-P11 performed better for almost all studied traits, it may be used as male to develop good quality petunia hybrids. It also indicated that in future breeding program of IAGS-P8, IAGS-P9 and IAGS-P11, these traits are important for primary selection to increase seed yield per plant of petunia under various environmental regimes. Moreover, this method proved to be more efficient as it reduced the cost, money, time and efficacy for better selection in petunia improvement program because the hybridization procedure of petunia is very difficult. When researcher take important traits like seed yield and flower size in account then there may be a chance to improve yield and quality of petunia. However, further studies are required which should cover different years and locations.

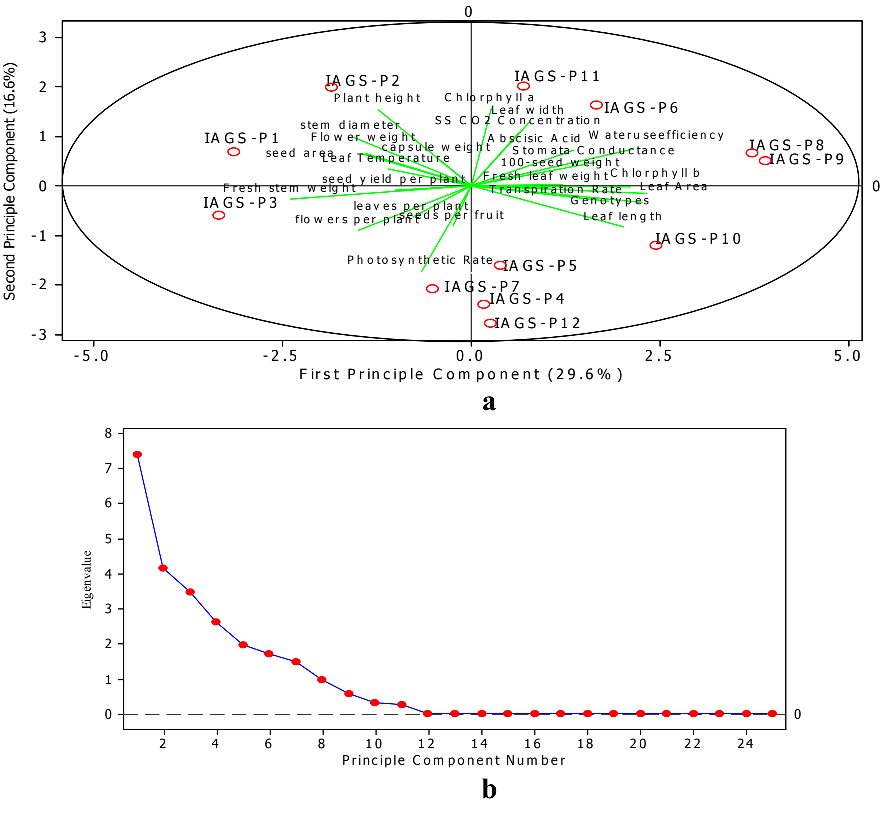
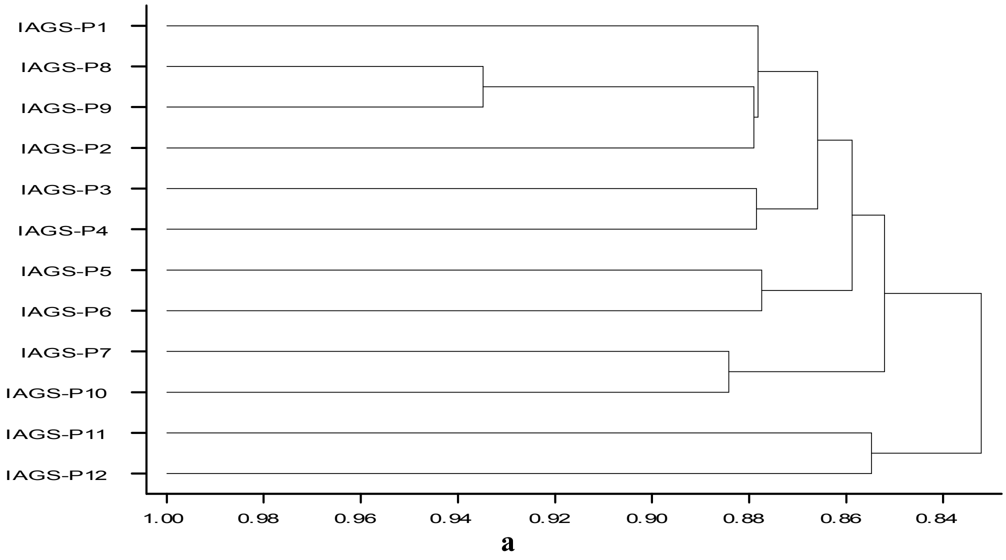


***Figure 3.*** ***a*** *Principle component analysis of yield and its attributing traits.* ***b*** *Scree plot and respective eigen values*

***Table 4.*** *Principal component analysis*

| **Eigen value** | 7.3977 | 4.1516 | 3.4767 | 2.6343 |
|---|---|---|---|---|
| **Proportion** | 0.296 | 0.166 | 0.139 | 0.105 |
| **Cumulative** | 0.296 | 0.462 | 0.601 | 0.706 |
| **Traits** | **PC1** | **PC2** | **PC3** | **PC4** |
| Photosynthetic rate | 0.689 | 0.415 | 0.016 | 0.055 |
| Leaf temperature | -0.15 | 0.083 | -0.336 | -0.093 |
| Chlorphyll a | 0.035 | 0.376 | 0.07 | 0.036 |
| Chlorphyll b | 0.286 | -0.005 | -0.025 | -0.217 |
| Stomata conductance | 0.221 | 0.118 | -0.297 | -0.047 |
| Transpiration rate | 0.313 | -0.037 | 0.151 | -0.162 |
| Sub-stomata CO2 concentration | 0.087 | 0.263 | 0.029 | 0.002 |
| Water use efficiency | 0.29 | 0.183 | 0.123 | -0.202 |
| Leaves per plant | -0.104 | -0.15 | -0.34 | 0.257 |
| Plant height | -0.166 | 0.371 | 0.137 | -0.033 |
| Stem diameter | -0.213 | 0.243 | -0.044 | -0.124 |
| Flowers per plant | -0.203 | -0.216 | 0.315 | -0.108 |
| Leaf length | 0.274 | -0.201 | -0.01 | 0.158 |
| Leaf width | 0.104 | 0.313 | 0.076 | 0.248 |
| Leaf area | 0.305 | -0.077 | 0.018 | 0.241 |
| Fresh leaf weight | 0.136 | -0.007 | -0.185 | -0.041 |
| Fresh stem weight | -0.324 | -0.064 | 0.176 | 0.002 |
| Flower weight | -0.191 | 0.16 | 0.018 | 0.337 |
| Seeds per fruit | -0.032 | -0.196 | 0.414 | -0.134 |
| 100-seed weight | 0.161 | 0.009 | -0.074 | 0.371 |
| Seed area | -0.199 | 0.162 | 0.082 | -0.106 |
| Seed yield per plant | -0.136 | -0.021 | -0.316 | -0.056 |
| Capsule weight | -0.062 | 0.135 | -0.205 | -0.499 |
| Abscisic acid | 0.184 | 0.178 | 0.336 | 0.075 |



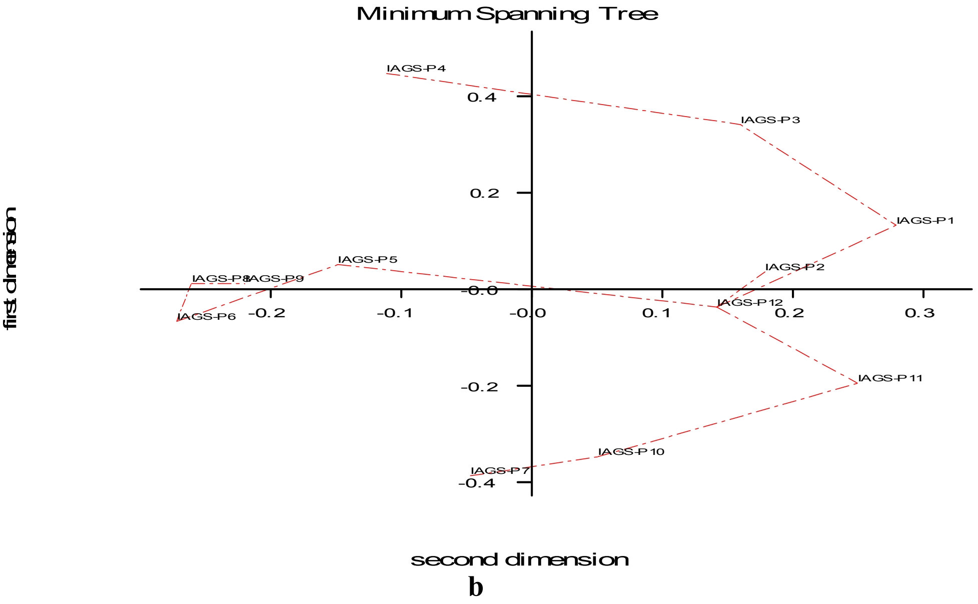


***Figure 4.*** ***a*** *Dendrogram analysis based on hierarchal clustering. Association of petunia lines on genetic basis of all studied traits.* ***b*** *Minimum spanning tree using egine values for petunia lines on the basis of all studied traits*

***Table 5.*** *Factor loadings for various traits of petunia*

| **Factor1** | **Factor loadings** | **% Communality** |
|---|---|---|
| Chlorophyll a | 0.603 | 36.2 |
| Chlorophyll b | 0.766 | |
| Stomata conductance | 0.564 | |
| Transpiration rate | 0.873 | |
| Water use efficiency | 0.970 | |
| leaves per plant | 0.659 | |
| stem diameter | 0.929 | |
| Leaf length | 0.887 | |
| Leaf area | 0.850 | |
| Seed yield per plant | 0.867 | |
| Abscisic acid | 0.836 | |
| Number of flowers per plant | 0.756 | |
| **Factor2** | | |
| Sub-stomata CO2 concentration | -0.545 | 27.1 |
| Plant height | -0.894 | |
| Fresh shoot weight | -0.628 | |
| **Factor3** | | |
| Seed size | 0.309 | 13.1 |
| Leaf length | 0.333 | |
| Leaf area | 0.307 | |
| Fresh leaf weight | 0.369 | |
| Seed per fruit | 0.354 | |
| **Factor4** | | |
| Photosynthetic rate | 0.211 | 9.40 |
| Capsule weight | 0.116 | |
| Leaf width | 0.129 | |
| stem diameter | 0.279 | |
| Leaf temperature | 0.217 | |
| Flower weight | 0.163 | |
| 100-seed weight | 0.296 | |
| Cumulative variance | | 85.80 |

## Conclusion

The present study was carried out to investigate the higher seed yield petunia line during the experimental seasons of 2011-2014. The data for various morphological, physiological and seed yield traits were recorded, analyzed and interpreted for final inferences. It was found that the lines IAGS-P8, IAGS-P9 and IAGS-P11 performed well. From correlation analysis, heritability, genetic advance, principle component analysis, factor analysis and regression analysis that stomata conductance, chlorophyll ‘a’ contents, chlorophyll ‘b’ contents, flowers per plant, flower fresh weight, seed area, seed weight and abscisic acid contributed higher to seed yield per plant in petunia. The higher abscisic acid contribution suggested that the petunia genotypes may be used to develop good seed yield per plant and large number of flowers per plant in petunia. The more research and experimentation should be carried out to improve yield and quality of petunia flower.

**Conflict of interests.** The authors declare the absence of potential conflict of interests.

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## APPENDIX

### *1. Photosynthetic rate*

*Genotypes mean homogeneous groups*

IAGS-P11 19.578 A
IAGS-P8 19.267 B
IAGS-P9 18.245 C
IAGS-P7 16.888 D
IAGS-P4 16.102 E
IAGS-P5 15.334 F
IAGS-P3 13.572 G
IAGS-P10 11.245 H
IAGS-P1 11.112 I
IAGS-P6 9.9033 J
IAGS-P2 9.1093 K
IAGS-P12 8.8883 L
Alpha 0.05 Standard Error for Comparison 9.393E-03

### *2. Leaf temperature*

*Genotypes mean homogeneous groups*

IAGS-P11 24.620 A
IAGS-P8 24.175 B
IAGS-P9 23.835 C
IAGS-P3 23.730 C
IAGS-P1 22.176 D
IAGS-P2 21.428 E

IAGS-P4 21.166 F
IAGS-P10 19.288 G
IAGS-P5 18.951 H
IAGS-P12 18.930 H
IAGS-P7 18.288 I
IAGS-P6 17.732 J
Alpha 0.05 Standard Error for Comparison 0.0613

### *3. Chlorphyll a*

*Genotypes mean homogeneous groups*

IAGS-P11 3.5467 A
IAGS-P12 3.5267 AB
IAGS-P9 3.5167 AB
IAGS-P8 3.4267 B
IAGS-P7 3.1867 C
IAGS-P2 3.1667 CD
IAGS-P3 3.0867 CDE
IAGS-P1 3.0867 CDE
IAGS-P4 3.0567 DE
IAGS-P5 3.0367 E
IAGS-P10 3.0067 E
IAGS-P6 2.7467 F
Alpha 0.05 Standard Error for Comparison 0.0579

### *4. Chlorphyll b*

*Genotypes mean homogeneous groups*

IAGS-P11 1.9267 A
IAGS-P9 1.9067 A
IAGS-P8 1.9067 A
IAGS-P6 1.8167 B
IAGS-P10 1.7567 B
IAGS-P12 1.5967 C
IAGS-P2 1.4167 D
IAGS-P4 1.4067 D
IAGS-P1 1.3667 DE
IAGS-P5 1.3067 EF
IAGS-P7 1.2667 F
IAGS-P3 1.2567 F
Alpha 0.05 Standard Error for Comparison 0.0386

### *5. Stomata conductance*

*Genotypes mean homogeneous groups*

IAGS-P11 0.0567 A
IAGS-P7 0.0433 AB
IAGS-P9 0.0400 BC
IAGS-P8 0.0367 BCD
IAGS-P10 0.0367 BCD
IAGS-P1 0.0267 CDE
IAGS-P12 0.0267 CDE
IAGS-P6 0.0267 CDE

IAGS-P3 0.0233 DE
IAGS-P2 0.0200 E
IAGS-P4 0.0167 E
IAGS-P5 0.0133 E
Alpha 0.05 Standard Error for Comparison 6.804E-03

### 6. *Transpiration rate*

*Genotypes mean homogeneous groups*

IAGS-P10 1.4567 A
IAGS-P11 1.4500 A
IAGS-P8 1.3933 B
IAGS-P9 1.2500 C
IAGS-P5 0.8633 D
IAGS-P1 0.8267 E
IAGS-P12 0.7733 F
IAGS-P7 0.7700 F
IAGS-P6 0.6833 G
IAGS-P2 0.5233 H
IAGS-P4 0.5167 H
IAGS-P3 0.0967 I
Alpha 0.05 Standard Error for Comparison 0.0176

### 7. *Sub-stomata $CO_2$ concentration*

*Genotypes mean homogeneous groups*

IAGS-P11 255.00 A
IAGS-P8 247.33 B
IAGS-P12 237.00 C
IAGS-P9 205.33 D
IAGS-P10 181.00 E
IAGS-P3 174.00 F
IAGS-P1 148.00 G
IAGS-P2 143.33 H
IAGS-P4 68.667 I
IAGS-P6 66.000 J
IAGS-P5 33.333 K
IAGS-P7 27.667 L
Alpha 0.05 Standard Error for Comparison 0.8165

### 8. *Water use efficiency*

*Genotypes mean homogeneous groups*

IAGS-P11 14.641 A
IAGS-P8 12.953 B
IAGS-P9 8.7000 C
IAGS-P7 8.2507 D
IAGS-P1 7.4390 E
IAGS-P6 6.3847 F
IAGS-P2 5.7450 G
IAGS-P10 5.0213 H
IAGS-P5 4.7317 H
IAGS-P12 4.2437 I

IAGS-P4 2.6817 J
IAGS-P3 0.7123 K
Alpha 0.05 Standard Error for Comparison 0.1471

### *9. Leaves per plant*

*Genotypes mean homogeneous groups*

IAGS-P9 91.333 A
IAGS-P11 91.333 A
IAGS-P8 90.333 B
IAGS-P2 89.333 C
IAGS-P7 88.333 D
IAGS-P4 87.333 E
IAGS-P12 86.333 F
IAGS-P6 85.333 G
IAGS-P5 84.333 H
IAGS-P3 83.333 I
IAGS-P10 82.333 J
IAGS-P1 81.333 K
Alpha 0.05 Standard Error for Comparison 0.4714

### *10. Plant height*

*Genotypes mean homogeneous groups*

IAGS-P11 62.357 A
IAGS-P9 62.247 A
IAGS-P8 61.127 B
IAGS-P3 60.247 C
IAGS-P4 59.997 D
IAGS-P12 59.347 E
IAGS-P5 56.797 F
IAGS-P7 52.367 G
IAGS-P10 51.947 H
IAGS-P6 49.017 I
IAGS-P1 47.357 J
IAGS-P2 47.017 K
Alpha 0.05 Standard Error for Comparison 0.0910

### *11. Stem diameter*

*Genotypes mean homogeneous groups*

IAGS-P11 0.5967 A
IAGS-P9 0.5733 B
IAGS-P8 0.5507 C
IAGS-P3 0.5480 D
IAGS-P6 0.5210 E
IAGS-P4 0.5120 F
IAGS-P2 0.5047 G
IAGS-P7 0.4930 H
IAGS-P12 0.4920 H
IAGS-P5 0.4550 I
IAGS-P10 0.4433 J
IAGS-P1 0.4420 J

Alpha 0.05 Standard Error for Comparison 1.217E-03

### *12. Flowers per plant*

*Genotypes mean homogeneous groups*

IAGS-P11 147.33 A
IAGS-P9 146.33 B
IAGS-P8 145.33 C
IAGS-P12 141.33 D
IAGS-P3 141.33 D
IAGS-P1 140.33 E
IAGS-P6 139.33 F
IAGS-P2 139.33 F
IAGS-P4 139.33 F
IAGS-P5 138.33 G
IAGS-P7 137.33 H
IAGS-P10 136.33 I
Alpha 0.05 Standard Error for Comparison 0.4714

### *13. Leaf length*

*Genotypes mean homogeneous groups*

IAGS-P8 7.000 A
IAGS-P11 6.9100 B
IAGS-P12 6.3400 C
IAGS-P9 6.1500 D
IAGS-P5 6.1000 E
IAGS-P6 6.0900 E
IAGS-P7 6.0400 F
IAGS-P2 5.8900 G
IAGS-P10 5.8900 G
IAGS-P4 5.2100 H
IAGS-P1 5.1600 I
IAGS-P3 5.1300 I
Alpha 0.05 Standard Error for Comparison 0.0216

### *14. Leaf width*

*Genotypes mean homogeneous groups*

IAGS-P11 1.1033 A
IAGS-P8 1.1033 A
IAGS-P9 1.1033 A
IAGS-P10 1.0833 AB
IAGS-P3 1.0633 ABC
IAGS-P1 1.0433 BCD
IAGS-P6 1.0333 BCD
IAGS-P5 1.0133 CDE
IAGS-P12 1.0033 DEF
IAGS-P4 0.9733 EF
IAGS-P2 0.9733 EF
IAGS-P7 0.9533 F
Alpha 0.05 Standard Error for Comparison 0.0249

### *15. Leaf area*

*Genotypes mean homogeneous groups*

IAGS-P11 7.423 A
IAGS-P8 6.603 B
IAGS-P9 6.107 C
IAGS-P12 5.847 CD
IAGS-P2 5.837 CDE
IAGS-P6 5.677 DE
IAGS-P7 5.667 DE
IAGS-P5 5.557 E
IAGS-P10 4.850 F
IAGS-P1 4.703 F
IAGS-P3 4.413 G
IAGS-P4 3.990 H
Alpha 0.05 Standard Error for Comparison 0.1392

### *16. Fresh leaf weight*

*Genotypes mean homogeneous groups*

IAGS-P9 0.7660 A
IAGS-P11 0.7120 AB
IAGS-P8 0.7100 AB
IAGS-P10 0.6920 B
IAGS-P7 0.6880 B
IAGS-P12 0.6840 B
IAGS-P5 0.6680 BC
IAGS-P3 0.6600 BC
IAGS-P4 0.6220 CD
IAGS-P2 0.5880 D
IAGS-P6 0.5760 D
IAGS-P1 0.4860 E
Alpha 0.05 Standard Error for Comparison 0.0294

### *17. Fresh stem weight*

*Genotypes mean homogeneous groups*

IAGS-P8 52.610 A
IAGS-P11 51.960 B
IAGS-P9 51.190 C
IAGS-P1 50.070 D
IAGS-P2 49.960 DE
IAGS-P12 49.940 E
IAGS-P6 49.940 E
IAGS-P4 49.940 E
IAGS-P3 47.740 F
IAGS-P5 47.440 G
IAGS-P10 45.410 H
IAGS-P7 44.490 I
Alpha 0.05 Standard Error for Comparison 0.0535

### *18. Flower weight*

*Genotypes mean homogeneous groups*

IAGS-P9 0.6633 A
IAGS-P11 0.6233 AB
IAGS-P3 0.6143 BC
IAGS-P8 0.6143 BC
IAGS-P4 0.6053 BC
IAGS-P6 0.5963 BCD
IAGS-P2 0.5943 BCD
IAGS-P10 0.5923 BCD
IAGS-P1 0.5903 BCD
IAGS-P5 0.5863 BCD
IAGS-P12 0.5763 CD
IAGS-P7 0.5593 D
Alpha 0.05 Standard Error for Comparison 0.0205

### *19. Seeds per fruit*

*Genotypes mean homogeneous groups*

IAGS-P11 872.33 A
IAGS-P9 871.33 B
IAGS-P8 870.33 C
IAGS-P1 867.33 D
IAGS-P12 867.33 D
IAGS-P10 866.33 E
IAGS-P2 866.33 E
IAGS-P5 865.33 F
IAGS-P5 864.33 G
IAGS-P4 863.33 H
IAGS-P7 862.33 I
IAGS-P3 857.33 J
Alpha 0.05 Standard Error for Comparison 0.4714

### *20. 100-seed weight*

*Genotypes mean homogeneous groups*

IAGS-P11 12.130 A
IAGS-P8 12.120 AB
IAGS-P9 12.110 BC
IAGS-P1 12.100 CD
IAGS-P7 12.090 DE
IAGS-P10 12.080 E
IAGS-P2 12.080 E
IAGS-P12 12.020 F
IAGS-P3 12.010 F
IAGS-P6 11.990 G
IAGS-P5 11.970 H
IAGS-P4 11.890 I
Alpha 0.05 Standard Error for Comparison 8.165E-03

### *21. Seed area*

*Genotypes mean homogeneous groups*

IAGS-P11 0.3753 A
IAGS-P9 0.3723 A
IAGS-P8 0.3693 AB
IAGS-P12 0.3683 ABC
IAGS-P1 0.3653 ABCD
IAGS-P2 0.3593 BCD
IAGS-P3 0.3583 CD
IAGS-P6 0.3563 DE
IAGS-P4 0.3463 EF
IAGS-P7 0.3433 F
IAGS-P5 0.3393 FG
IAGS-P10 0.3323 G
Alpha 0.05 Standard Error for Comparison 5.249E-03

### 22. *Seed weight*

*Genotypes mean homogeneous groups*

IAGS-P11 116.20 A
IAGS-P4 115.98 B
IAGS-P8 115.85 C
IAGS-P9 115.40 D
IAGS-P12 115.32 D
IAGS-P7 115.32 D
IAGS-P6 115.31 D
IAGS-P10 115.06 E
IAGS-P1 114.87 F
IAGS-P2 114.32 G
IAGS-P3 114.26 G
IAGS-P5 113.63 H
Alpha 0.05 Standard Error for Comparison 0.0618

### 23. *Abscisic acid*

*Genotypes mean homogeneous groups*

IAGS-P11 0.1250 A
IAGS-P8 0.1240 AB
IAGS-P9 0.1240 AB
IAGS-P10 0.1230 AB
IAGS-P3 0.1230 AB
IAGS-P1 0.1220 AB
IAGS-P12 0.1210 B
IAGS-P4 0.1140 C
IAGS-P5 0.1110 CD
IAGS-P6 0.1100 D
IAGS-P7 0.1090 D
IAGS-P2 0.1030 E
Alpha 0.05 Standard Error for Comparison 1.633E-03

### 24. *Seed yield per plant*

*Genotypes mean homogeneous groups*

IAGS-P11 54.153 A
IAGS-P9 53.353 B

IAGS-P8 52.263 C
IAGS-P6 52.143 D
IAGS-P2 51.263 E
IAGS-P5 51.263 E
IAGS-P10 50.153 F
IAGS-P7 49.593 G
IAGS-P1 48.923 H
IAGS-P12 48.703 I
IAGS-P3 46.263 J
IAGS-P4 43.603 K
Alpha 0.05 Standard Error for Comparison 0.0249