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Journal of Applied Research on Medicinal and Aromatic Plants

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Path and factor analysis of roselle (Hibiscus sabdariffa L.) performance



Hamid-Reza Fallahi^{a,*}, Seyyed Hamid Reza Ramazani^a, Morteza Ghorbany^b, Mahsa Aghhavani-Shajari^c

^a Department of Agronomy and Plant Breeding, Faculty of Agriculture, University of Birjand, Birjand, Iran

^b Department of Biology, Faculty of Science, University of Birjand, Birjand, Iran

^c Department of Agronomy, Ferdowsi University of Mashhad, Mashhad, Iran

ARTICLE INFO

Keywords: Correlation Medicinal plant Path analysis Sepal yield Stepwise regression

ABSTRACT

Roselle is known for delicacy and also for medicinal properties. There is only limited information available on genetics and breeding of its economic traits. In this experiment, roselle were grown as a split–split plot based on a randomized complete-block design with three replications during 2015, at the experimental station of University of Birjand in Sarayan, Iran. Treatments consisted of three factors, including irrigation management (two levels), humic-acid application (two levels) and mycorrhizal inoculation (three levels). Sixteen related traits were recorded. After data collection, correlation analysis, regression and path analysis (using the PATH software package) were performed. Analysis of variance showed that most of the studied traits were significantly affected by experimental factors. Based on stepwise regression, the biological yield, harvest index and sepal yield per plant were entered into the regression model in the last step (coefficient of determination = 96.8%). Path analysis showed that biological yield had the largest direct and positive impact on sepal production. Harvest index had a positive direct and negative indirect effect (through its negative relationship with sepals yield per plant and biological yield) on sepal yield per hectare. Finally, sepal yield per plant had a small, negligible positive direct effect, but its indirect effect (through the reduction of harvest index and increasing biological yield) was quite large on sepals yield per hectare. Therefore, biological yield and sepal yield per plant, can be used as indicators for selection for yield in roselle.

1. Introduction

Roselle (*Hibiscus sabdariffa* L.) is an annual industrial and medicinal herb, grows mainly in tropical and sub-tropical areas. This crop probably originated from West Africa or India but is currently grown in many regions of the world, such as parts of Asia, Central America and Australia (Babatunde and Mofoke, 2006; Futuless et al., 2010; Rahbarian et al., 2011; Sonar et al., 2013). In Iran, roselle is mainly produced in Sistan and Balouchestan province on about 300 ha with a mean dry calyx yield of 700–900 kg ha⁻¹. Roselle is cultivated for its stem fibers, leaves, seeds and especially for its edible calyces with the aim of preparing refreshing beverages, jellies and as a natural coloring agent (Fasoyiro et al., 2005a; Futuless et al., 2010; Sonar et al., 2013; Satyanarayana et al., 2015).

Roselle fruits (calyx) are containing many essential nutrients such as vitamin A, vitamin C, minerals, polysaccharide, pectin, β -carotene, anthocyanin and dietary fiber, also contains alkaloids, ascorbic acid, anisaldehyde, β -sitosterol, citric-acid, cyanidin-3-rutinoside,

delphinidin, galactose, gossypetin, hibiscetin, mucopolysaccharide, protocatechuic acid, quercetin, stearic acid and wax (Fasoyiro et al., 2005b; Hirunpanich et al., 2005). The approach of roselle is equally significant in alternative system of medicine as well as in conventional system of medicine. It is known to have anti-scorbutic, anti-diabetic and anti-hypertensive effects and so is emollient, diuretic, refrigerant, and sedative. The plant products (*viz.*, calyx, leaves, oil extracted from seeds) is also reported to be antiseptic, aphrodisiac, astringent, cholagogue, demulcent, digestive, purgative and resolving. In addition, it is used as a folk remedy in the treatment of abscesses, bilious conditions, cancer, cough, debility, dyspepsia, fever, hangover, heart ailments, hypertension, and neurosis (Hirunpanich et al., 2005; Da-Costa-Rocha et al., 2014).

The relationship between traits with yield is important, but to calculate the correlation coefficients did not specify the nature of the characteristics and uses of path analysis allowed the identification of direct and indirect effects of traits there. For this purpose, plant breeders are used path analysis as a tool to determine the effective traits in

http://dx.doi.org/10.1016/j.jarmap.2017.04.001 Received 23 July 2016; Received in revised form 29 March 2017; Accepted 2 April 2017 Available online 13 June 2017 2214-7861/ © 2017 Elsevier GmbH. All rights reserved.

^{*} Corresponding author at: Department of Agronomy and Plant Breeding, Faculty of Agriculture, University of Birjand, Birjand, South Khorasan Province, Iran. Tel.: + 98 9363574026. *E-mail addresses*: Hamidreza.fallahi@birjand.ac.ir (H.-R. Fallahi), Hrramazani@birjand.ac.ir (S.H.R. Ramazani), mghorbany@birjand.ac.ir (M. Ghorbany), Mahsa.aghavanishajari@stu.um.ac.ir (M. Aghhavani-Shajari).

Table 1

The main climatic indices of experimental site during experiment.

| Growth month | Precipitation (mm) | Potential evaporation (mm)* | Monthly average humidity (%) | Monthly sunshine (h) | Average of minimum temperatures (°C) | Average of maximum temperatures (°C) |
|--------------|--------------------|--------------------------------|---------------------------------|-------------------------|---|---|
| April | 12 | 135.8 | 38 | 248.5 | 11.7 | 25.1 |
| May | 5.3 | 297.2 | 26 | 287.6 | 17.1 | 30.8 |
| June | 0 | 417.6 | 16 | 344.1 | 21.1 | 35.8 |
| July | 0 | 479.0 | 16 | 355.6 | 24.2 | 37.5 |
| August | 0 | 418.9 | 16 | 368.1 | 21.5 | 35.5 |
| September | 0 | 304.3 | 22 | 343.9 | 16.8 | 32.2 |
| October | 1.9 | 216.1 | 27 | 288.4 | 14.4 | 29.0 |
| November | 9 | 97.7 | 45 | 206.8 | 8.3 | 20.2 |

These data were used for determination of irrigation dates in treatments of normal (irrigation after 100 mm pan evaporation) and deficit irrigation (irrigation after 200 mm pan evaporation). The amount of used water in each irrigation time was $600 \text{ m}^3 \text{ ha}^{-1}$.

yield. Path analysis has been proposed by Wright (1921), a method in which the relationship between the characters and their direct and indirect effects is clarified. Path analysis is a method that reveals the relationships between traits and their direct and indirect effects on performance. This method requires the identification of casual relationships among traits (Allah-Gholipour, 1997). The simple correlation coefficient, does not provide an accurate opinion of the importance of direct and indirect effects of each yield components (Rafeie and Saeidi, 2005). Moreover, since the number of traits that have a negative correlation with respect to complex traits together, final judgment cannot be made solely on the basis of simple correlation coefficients (Tousi-Mojarad and Bihamta, 2007). Also, often a trait, in addition to direct effects on some traits, have an effect on these indirectly via other traits. In this case, path analysis (particularly sequential path coefficients analysis) method determine the share of direct and indirect effects on other traits (Rezaei and Soltani, 1998; Rafeie and Saeidi, 2005).

Environmental conditions and genotype interaction affected the relationships among plant characters. Correlation and path analysis are the two best approaches to determine these relations (Dalkani et al., 2011) that has been used by many researchers in different crops including many medicinal plants (Chitra and Rajamani, 2010; Karuppaiah and Senthil Kumar, 2010; Bardideh et al., 2013). So far, in some studies the path and factor analysis and genetic improvement of roselle has been investigated (Ibrahim et al., 2013a; Sabiel et al., 2014), but there is a few attentions and limited information regarding its genetics, breeding and production (Sabiel et al., 2014). Therefore, the aim of this study was determine the sepal yield-related characters and their relationships with others for determination of the best criteria for high sepal yield condition for screening, using factor and path analysis and separate the relationship between sepal yield and its components into direct and indirect effects and distinguish the cause and effect relationships between them.

2. Material and methods

For evaluation of the sepal yield-related criteria in roselle and their relationships with others for determination of the best index for high yield condition screening, using factor and path analysis, an experiment was conducted during 2015. Roselle plants were grown as a split–split plot based on a randomized complete block design with three

replications, in experimental station of Sarayan Faculty of Agriculture (33° N, 58° E and 1450 msl), University of Birjand, Iran. The experimental site has semi-arid climate with an average annual precipitation and mean annual temperature of 110 mm and 17 °C, respectively. The main climatic indices of experimental site during study are presented in Table 1.

Experimental treatments were contained three factors including irrigation with two levels (normal and deficit irrigation: irrigation after 100 and 200 mm pan evaporation, respectively), humic-acid with two levels (0 and 4 kg ha⁻¹) and mycorrhizal inoculation with three levels (Glomus versiforme, Glomus intraradices and no-inoculation). Water regime was considered as the main plots, humic acid application levels were sub-plots and mycorrhizal inoculation treatments were sub-subplots. There were three replications in experiment with 12 plots per replicate (Each plot had an area of 4 m²). Mycorrhizal species were achieved from TuranBiotech company (Turanbiotch.ir), which were prepared by trap culture method on berseem clover (Trifolium alexandrinum L.). Mycorrhizal fungi according to the manufacturer's recommendation were used under the planted seeds at the rate of 2 g per plant. The used humic acid was from Brand of Humixtract, produced in Spain. Its total humic extract, humic acids, polycarboxilic acid, potassium oxide and calcium oxide were 70, 38, 32, 10 and 1% W/W Total, respectively. Humic acid was used through irrigation water two times during vegetative growth (15 and 35 days after emergence).

Manual seed planting (using 'Saravan' cultivar as a local accession) was carried out on 20th April, 2015 with density of 20 plant per m⁻² (10 × 50 cm intra- and inter-row distances). The main physical and chemical properties of experimental site with respect to soil are shown in Table 2. All plots were irrigated similarly two times during the first week after seed sowing and then irrigation treatments were done separately in all plots belonging two different irrigation regimes until November 15 when irrigation was stopped. The amount of used water in each irrigation time was 600 m³ ha⁻¹. Irrigation was stopped two weeks before roselle fruits were harvested.

At the end of growth cycle, for measuring of some morphological indices and yield components of roselle, five plants were selected randomly in each plot on 20th November, 2015. The selected plants were cut from above soil and then the amounts of mean plant height, number of lateral branches per plant, number of fruits per plant, mean fresh weight of fruit, leaf area, leaf dry weight, plant dry weight and sepals

Table 2

Main physical and chemical properties of experimental site with respect to soil.

| EC† (mS cm ⁻¹) | pH | O.C. ‡ (%) | N _{total} (%) | P _{ava} (%) | K _{ava} (%) | Sand (%) | Silt (%) | Clay (%) | Soil texture |
|----------------------------|------|------------|------------------------|----------------------|----------------------|----------|----------|----------|--------------|
| 2.27 | 8.49 | 0.13 | 0.016 | 0.0002 | 0.019 | 48.5 | 22.5 | 29 | Loam |

 $^{\dagger}EC = electrical conductivity.$

^{*}O.C = organic carbon.

| Source of variation | đf | Anthocyanin content (mg l ⁻¹) | Percentage of mycorthizal symbiosis | Vitamin C content (mg 100 g ⁻¹) | WUE _b (kg kg ⁻¹) † | WUE _s (kg kg ⁻¹) ‡ | Harvest index (%) | Biological yield (kg ha ⁻¹⁾ | Sepal yield (kg ha ^{– 1}) | Biological yield (g plant ⁻¹) | Leaf weight (g plant ⁻¹) | Leaf area (cm ² plant ⁻¹) | Sepal yield (g plant ⁻¹) | Fruit weight (g) | Number of fruit per plant | Number of branches | Plant height (cm) |
|--------------------------------------|-----------|---|---|---|--|---|-------------------------|--|--|---|---|---|---|------------------------|---------------------------------|-----------------------|-------------------------|
| Replication Irrigation management | 1 2 | 5.08 349.88 | 36.6 6.2 | 81016.7 [*] 70081.1 [*] | 0.015 0.000 | 1.58E-06 3.60E-05 | 0.7 0.5 | 888558 11911903- 4** | 31.0 62633 ^{**} | 65.10 [°] 2928.6 ^{**} | 2.08 40** | 7398.7 216380 [°] | 0.20 11.38** | 0.13 2.05* | 0.8 797.8** | 12.07* 29.6** | 3022.3 6000.6 |
| Error A | 2 | 84.93 | 15.7 | 1117.60 | 0.006 | 8.83 | 0.5 | 227262 | 434.8 | 1.18 | 0.14 | 9101.2 | 0.03 | 0.04 | 5.8 | 0.16 | 1492.5 |
| Humic acid application | 1 | 894.7** | 3306.2^{**} | 381477^{**} | 0.002 | 1.30E-05 | 0.1 | 2501775 | 74.0 | 14.57 | 7.83 | 59130.0^{*} | 0.00 | 0.17 | 0.4 | 1.22 | 4.94 |
| Irrigation \times Humic | 1 | 4396.4^{**} | 17.36 | 865.05 | 0.1^{**} | 0.00018* | 0.2 | 13306201^{*} | 13194^{*} | 184.51 | 5.81 | 45582.2^{*} | 0.00 | 0.12 | 34.5* | 0.79 | 1402.5 |
| Error B | 4 | 19.67 | 25.89 | 14112.05 | 0.007 | 1.26E-05 | 0.2 | 955949 | 1349.1 | 39.21 | 3.15 | 2850.2 | 0.02 | 0.03 | 4.1 | 3.29 | 340.9 |
| Mycorrhizal inoculation | 7 | 423.6** | 5725** | 743722^{**} | 0.2^{**} | 0.0001** | 0.1 | 19593968- ** | 9624** | 436.8** | 28.1** | 150348^{**} | 0.87** | 0.03 | 136.0^{**} | 10.8^{*} | 870.0 |
| Irrigation \times Mycorrhiza | 2 | 565.01** | 75.0 | 200470^{**} | 0.1^{**} | 0.0001^{**} | 1 * | 9686610** | 9538** | 228.5** | 11.46 | 63097** | 1.71^{**} | 0.23 | 80.2^{**} | 3.41 | 112.5 |
| Humic \times Mycorrhiza | 7 | 89.6 | 108.3 | 391226^{**} | 0.1^{**} | 1.52E-05 | 1^* | 12259455- ** | 1979^{*} | 394.8** | 22** | 48082^{**} | 0.07 | 0.22 | 140.6** | 4.33 | 129.8 |
| Irrigation × Humic × Myc- orrhiza | 7 | 126.8 | 44.4 | 778768** | 0.1^{**} | 0.000** | 1^* | 9228078** | 4643** | 283.8** | 7.54 | 3496.6 | 0.01 | 0.39* | 22.22* | 0.88 | 185.4 |
| Residual error | 16 | 35.8 | 32.1 | 20963.28 | 0.01 | 6.43E-06 | 0.2 | 820729 | 428.9 | 19.9 | 3.33 | 7644.9 | 0.06 | 0.09 | 3.9 | 2.21 | 329.51 |
| Total | 35 | 253.79 | 455.91 | 149626.6 | 0.037 | 3.18E-05 | 0.4 | 7304295 | 4018.9 | 183.54 | 7.53 | 29081.7 | 0.52 | 0.17 | 48.1 | 4.10 | 733.4 |
| C.V. | | 12.47 | 00.6 | 7.15 | 14.18 | 14.9 | 17 | 16.33 | 13.8 | 16.47 | 22.6 | 14.25 | 20.32 | 32.11 | 13.34 | 34.40 | 15.11 |
| * and ** are significantly c | lifferent | at $\alpha = 0.05$ and | $d \alpha = 0.01$, respectively. | ctively. | | | | | | | | | | | | | |

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dry weight per plant were determined. The samples were air-dried at ambient temperature (20–25 °C, in the shade) for 1 month. One week after that, the fruits of remained plants were harvested separately in each plot for measurement of biological (weight of all aerial parts) and economical yields (calyx weight) as well as harvest index calculation. Also, for determination of water use efficiency based on sepals and biological yields (WUE_s and WUE_b, respectively), the values of yields were divided on the amount of used water during plant growth. In addition, total anthocyanin content was measured using the pH-differential method as described by Swain (1965). Horwitz (1980) method was used for determination of ascorbic acid (vitamin C) content in sepals. Moreover, the method presented by Shirzad and Ghorbany (2015) was used for measurement of mycorrhizal frequency.

According to above description, sixteen related traits were recorded in this experiment. After data collection, correlation analysis, regression and path analysis were performed. The mean observation for each trait was used for statistical analysis. The analysis of variance, was used SAS-9.0 software (SAS Institute, 2002), regression analysis and factor analysis used Minitab-17.0 software (Minitab Inc., 2014, Pennsylvania, USA) and to determine important characteristics affecting the performance of the sepals used path analysis with PATH software (Garcia de Moral et al., 2005).

3. Results and discussion

3.1. Growth, yield and fruit quality

Effect of experimental factors (irrigation management, humic acid application and mycorrhizal inoculation) was significant on most qualitative and quantitative indices of roselle (Table 3). The details of means comparison have been previously presented in Fallahi et al. (2017a, b). Based on presented results, deficit irrigation decreased the morphological indices and vield components of roselle. However, mycorrhizal inoculation especially using G. intraradices and partially humic acid application reduced the negative effects of drought stress on growth and yield of roselle. Calyx yield for G. intraradices, G. versiforme and no-inoculation treatments, in 200 mm pan-evaporation was 130, 127 and 66 kg ha⁻¹, respectively. In addition, normal irrigation combined with humic acid application increased anthocyanins (67.1 mg l^{-1}) and vitamin C content (2177 mg 100 g⁻¹) over the control, which had lower anthocyanins (38.8 mg l^{-1}) and vitamin C content (1882 mg 100 g^{-1}). Humic acid application and mycorrhizal inoculation, especially using G. intraradices, showed the highest values of anthocyanins (56.9 mg l^{-1}) and vitamin C (2309 mg 100 g⁻¹) content. Furthermore, the most root mycorrhizal frequency was gained at normal irrigation \times humic application \times G. intraradices (95%) which was 65% more than that observed at control treatment (deficit irrigation \times no-humic \times no-mycorrhiza). Overall, application of humic acid and mycorrhizal inoculation evaluated as two useful strategies for roselle production in areas affected by drought stress.

3.2. Analysis of variance

Analysis of variance showed that there is a no significant difference between replications at 5% level of probability except in the case of vitamin C, biological yield per plant and number of branches per plant. Humic acid application had a significant effect on qualitative traits as anthocyanin, mycorrhizal symbiosis percentage, vitamin C (at 1%), as well as leaf area (at 5%). In addition, irrigation management had a significant effect on all traits except anthocyanin content, mycorrhizal symbiosis, water use efficiency, harvest index and plant height. Mycorrhizal inoculation also exerted a significant effect on most of qualitative and quantitative indices of roselle except harvest index, fruit weight and plant height (Table 3). In total, roselle growth, yield and quality affected considerably by all experimental factors. So far, the positive effect of accurate deficit irrigation and reducing effects of sever water stress has been reported on growth, sepal yield and quality of

Analysis of variation (mean of squares) of different traits in roselle influenced with irrigation, humic acid and mychoriza treatment

Table 3

water use efficiency based on biological yield

based on sepal yield.

use efficiency

= water

†WUE_b ‡WUE_s

Table 4

Loading of the first three most principal from factor analysis of different traits. .

| Variable | Factor1 | Factor2 | Factor3 |
|---|--------------|--------------|---------------|
| Anthocyanin content (mg l ⁻¹) | 0.21 | 0.22 | -0.60 |
| Vitamin C (mg 100 g^{-1}) | 0.09 | 0.15 | -0.65 |
| WUE_b (kg kg ⁻¹) | 0.74 | 0.38 | 0.13 |
| WUE _s (kg kg ⁻¹) | 0.48 | 0.77 | 0.36 |
| Mycorrhizal frequency (%) | 0.40 | 0.47 | <u>-</u> 0.33 |
| Harvest index (%) | <u>-0.24</u> | 0.60 | 0.43 |
| Biological yield (kg ha ⁻¹) | 0.96 | <u>-0.13</u> | 0.07 |
| Sepal yield (kg ha ⁻¹) | 0.90 | 0.10 | 0.24 |
| Biological yield per plant (g) | 0.95 | <u>-0.17</u> | 0.02 |
| Leaf weight (g plant $^{-1}$) | 0.84 | 0.12 | <u>-0.05</u> |
| Leaf area (cm^{-2} plant ⁻¹) | 0.90 | 0.12 | <u>-0.04</u> |
| Sepal yield per plant (g) | 0.83 | <u>-0.31</u> | <u>-0.09</u> |
| Fruit weight (g) | 0.53 | -0.63 | 0.23 |
| Number of fruit per plant | 0.92 | <u>-0.12</u> | <u>-0.03</u> |
| Number of lateral branches | 0.72 | 0.02 | <u>-</u> 0.46 |
| Plant height (cm) | 0.53 | <u>-0.17</u> | 0.44 |
| Var % | 0.49 | 0.13 | 0.11 |
| Cumulatively | 0.49 | 0.69 | 0.80 |
| | | | |

roselle (El-Boraie et al., 2009; Rahbarian et al., 2011). Some studies also confirm that humic acid (Sanjari-Mijani et al., 2015) and mycorrhizal inoculation (Sonar et al., 2013; Sembok et al., 2015) can improve the growth, yield and qualitative indices of sepals especially under environmental stress like drought.

3.3. Correlation between traits

The phenotypic correlation for traits measured using Pearson correlation coefficients (Table 5). There was a significant positive correlation between plant height, number of branches per plants, fruit number per plants, fruit weight, leaf area, leaf dry weight, biological yield and WUE_b with sepal yield per plant. In similar study on roselle Sabiel et al. (2014) concluded that calyx yield was significantly and positively correlated with number of branches per plant, number of capsules per plant, hay weight and plant height. Similar results were obtained by Ibrahim et al. (2013a) which reported that the close association between calyx yield and most of its components, at both phenotypic and genotypic levels over the two growth cycles may be attributed to genetic effects rather than environmental ones. Singh et al. (2015) concluded that roselle calyx yield is positively correlated with days to 50% flowering followed by plant height and branches per plant. Ibrahim and Hussein (2006) also from the positive correlation between number of fruit per plant with number of branches and plant height concluded that these traits may be good selections for improving seed and calyx yield in roselle.

3.4. Factor analysis

Factor analysis was used for determination of high heritability traits for screening of premier genotypes. Since no test of significant was performed for factor selection, the decision was rather arbitrary as to how magnitude of loading coefficient a variable should possess to be considered meaningful. Factors with values more than 1.0 were retained and whose more than 0.6 were be major (Acquaah et al., 1992). In this study, with this technique divided the 16 variables into 3 factors. These factors explained 80% of the total variation (Table 4). Factor 1 was associated with number of lateral branches, number of fruit, sepal yield per plant, leaf area per plant, leaf weight per plant, biological yield per plant, sepal yield ha⁻¹, biological yield ha⁻¹ and WUE_b with positive signs (0.72, 0.92, 0.83, 0.90, 0.84, 0.95, 0.90, 0.96 and 0.74, respectively) that explain 49% of total variation. The sign of the loading shows the direction of the relationship between the factor and the variable. Thus two traits with high magnitude in the same factor would be have high correlation (Seiler and Stafford, 1985). Factor 2 was associated with WUE_s, harvest index and fruit weight (0.77, 0.60 and -0.63, respectively). Factor 3 was associated with anthocyanin and vitamin C with negative signs (-0.60 and -0.65, respectively) that explain 11% of total variation. This means with increasing sepal yield ha⁻¹, decreasing of qualitative traits occur. Thus we could name factor 1 as "quantitative factors" and factor 3 as "qualitative factors".

3.5. Regression equations between sepal yield and other traits

Using stepwise regression model (with 15% probability of entrance to model and 2% probability of transition from model), traits with no significant or low significant were excluded from the model. The results showed that the biological yield, harvest index and sepal yield per plant were entered in regression model in the last step with 96.83% of coefficient of determination (Table 6). If we consider the sepal yield per ha = Y, Biological yield per ha = X1, Harvest index = X2 and Sepal yield per plant = X3, then the overall equation for step by-step will be as follows: Y = $-101.24 + 0.023X_1 + 42.2X_2 + 6.6X_3$. As we can see, all the attributes of X1–X3 with positive factors have an impact on production of sepals. In similar study Ibrahim et al. (2013a) concluded that number of fruiting branches per plant, number of capsules in main stem and fruit weight can be used as selection criteria for the improvement of calyx yield per plant in roselle.

3.6. Path analysis between traits and sepal yield

Path analysis was conducted for sepal yield ha^{-1} as the dependent variable and the remaining three selected traits in the regression model (biological yield, harvest index and sepal yield per plant) as the independent variables. Path analysis was recorded in Table 7 and Fig. 1. Results showed that biological yield had the most direct and positive impact on sepal production. Harvest index had a positive direct and negative indirect effect (through decreasing the sepals yield per plant and biological yield) on increasing sepal yield ha⁻¹. Finally, sepals vield per plant had a positive direct and negative indirect effect (through the reduction of harvest index and increasing biological yield) on enhancement of sepals yield per hectare. It has been reported that if there is a direct correlation between yield and one index, this shows a real relation between them and can select that index for yield breeding programs. However, if there is an indirect correlation between a criterion with yield through second index, the selection must be done on the second ones (Nasri et al., 2012). Accordingly, similar to finding of Mehrabadi et al. (2015) on cotton biological yield is the superior trait to be selected for yield increasing in roselle. In similar study on roselle path coefficient analysis showed that number of branches per plant had a positive direct effect on dry calyx yield. In addition, it was concluded that number of branches per plant, number of capsules per plant, hay weight and plant height would be the best selection criteria for roselle improvement (Sabiel et al., 2014). In another study on roselle the path analysis indicated that fruit weight had the highest direct effect (0.46) on calyx yield per plant, while fruit yield had the lowest one (-0.19)(Ibrahim et al., 2013a). Singh et al. (2015) also observed that the largest direct contribution to roselle seed yield was that of plant height and calyx yield. In another study on roselle, it was concluded that due to low heritability of calyx yield per plant, the indirect selection through its components assumes important (Ibrahim et al., 2013b).

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| ion between different critria in study of irrigation management, humic acid |
| ttion between different critria in study of irrigation management, humic acid |

| Table 5 Correlation betwee | en different crit | ria in study o | f irrigation ma | anagement, | humic acid a | tpplication a | nd mycorrhiz | al inoculatio | n effects on r | oselle sepals y | ield. | | | | | |
|--------------------------------------|-------------------|----------------------------------|---------------------------------|---------------------|----------------------------------|--|-------------------------------------|-------------------------|--|---|----------------------|------------------------------|--|--|---|--|
| Traits | Plant height | Number of lateral branches | Number of fruit per plant | Fruit weight (g) | Sepals yield per plant (g) | Leaf area per plant (cm ²) | Leaf dry weight per plant (g) | Plant dry weight (g) | Sepals yield (kg ha ⁻¹) | Biological yield (kg ha ⁻¹) | Harvest index (%) | Mycorrhizal frequency (%) | WUE _s (kg kg ⁻¹) | WUE _b (kg kg ⁻¹) | Vitamin C content (mg 100 g ⁻¹) | Anthocyanin content (mg l ⁻¹) |
| Plant height | 1 | | | | | | | | | | | | | | | |
| number of lateral branches | /00.0 | T | | | | | | | | | | | | | | |
| Number of fruit | 0.47** | 0.644** | 1 | | | | | | | | | | | | | |
| per plant | | | | | | | | | | | | | | | | |
| Fruit weight | 0.50** | 0.264 | 0.515** | 1 | | | | | | | | | | | | |
| Sepals yield per | 0.47** | 0.613^{**} | 0.829** | 0.59** | 1 | | | | | | | | | | | |
| plant | | | | | | | | | | | | | | | | |
| Leaf area per | 0.300^{*} | 0.685** | 0.786** | 0.329* | 0.711^{**} | 1 | | | | | | | | | | |
| plant I موطح معد بينمانطية | 0 334 | 0 688** | 0 686** | 0.383* | 0 638** | 0.880** | - | | | | | | | | | |
| ner nlant | 107.0 | 000.0 | 000.0 | 0.07.0 | 070.0 | 600.0 | - | | | | | | | | | |
| Dlant day waight | 0 61 ** | **0990 | ** 100 0 | **090 | **000 | 0.015** | **9920 | - | | | | | | | | |
| Plant ary weight Senals vield ha | TC.U | 0.527** | 0.840** | 0.45** | 0.746** | C18.0 | 0.715** | L 0.85** | - | | | | | | | |
| Biological | 0.56** | 0.616** | 0.922** | 0.55** | 0.803** | 0.837** | 0.770** | 0.96** | 0.90** | 1 | | | | | | |
| yield ha | | | | | | | | | | | | | | | | |
| Harvest index | -0.044 | - 0.298 | -0.251 | -0.35^{*} | -0.248 | -0.181 | -0.183 | -0.316 | 0.109 | -0.297 | 1 | | | | | |
| Mycorrhizal | 0.154 | 0.329^{*} | 0.334* | -0.048 | 0.236 | 0.308 | 0.235 | 0.273 | 0.241 | 0.282 | -0.100 | 1 | | | | |
| frequency | | | | | | | | | | | | | | | | |
| WITE_ | 0.266 | 0.205 | 0.312 | 0.083 | 0.088 | 0.492.** | 0 448** | 0.315 | 0.57** | 0.364* | 0 471** | 0 4 27 ** | - | | | |
| WUE | 0.354* | 0.461** | 0.589** | 0.190 | 0.333* | 0.721^{**} | 0.671** | 0.64** | 0.60** | 0.688** | -0.202 | 0.479** | 0.75** | 1 | | |
| Vitamin C content | -0.103 | 0.227 | 0.095 | -0.123 | 0.088 | 0.105 | -0.000 | 0.021 | 0.045 | 0.072 | -0.149 | 0.271 | -0.040 | 0.023 | 1 | |
| Anthocyanin | -0.006 | 0.416** | 0.197 | -0.105 | 0.240 | 0.144 | 0.195 | 0.172 | 0.122 | 0.099 | 0.023 | 0.309 | 0.041 | 0.008 | 0.27 | 1 |
| content | | | | | | | | | | | | | | | | |
| * and ** are signif | ïcantly differen | tt at $\alpha = 0.05$ | and $\alpha = 0.01$ | l, respective | ly and ns is | non-significa | mt. | | | | | | | | | |

Table 6

Summary of linear stepwise regression analysis for sepal yield as functional variable and others as independent variable in roselle.

| Variable added to the model | Stepwise reg | ression procedures | |
|--|--------------|--------------------|--------------|
| | 1 | 2 | 3 |
| Constant Biological yield (kg ha ^{-1}) | 32.42 | -100.96 | -101.24 |
| Harvest index (%) | 0.021 | 42.1** | 42.2** |
| Sepal yield per plant (g) Determination coefficient | 80.7 | 96.71 | 6.6 96.83 |

Table 7

Direct and indirect and residual effects of different traits on roselle sepals yield.

| Indirect effects via | Direct effects | | |
|--|--|---|-------------------------------|
| | Sepals yield per plant (g) 0.074 | Biological yield (kg ha ^{−1}) 0.964 | Harvest index (%) 0.413 |
| | | | |
| Sepais yield per plant (g) Biological yield (kg ha ⁻¹) Harvest index (%) | 0.774 - 0.103 | 0.059 | -0.019 -0.287 |



Fig. 1. Path diagram showing impacts of sepal yield per plant (Trait 1), biological yield per ha (Trait 2), and harvest index (Trait 3) on sepal yield per ha (Trait 4). (*P*) Path coefficient, (*r*) correlation coefficient and (*R*) Residual effects.

4. Conclusion

The path analysis shows that biological yield was the highest direct effect on sepals yield that is similar to Nemati-Lafmejani et al. (2011) for *Rosa damascena* flower performance. Biological yield had a significant positive correlation with sepal yield per ha ($r = 0.90^{**}$), number of fruit per plant ($r = 0.92^{**}$) and plant dry weight ($r = 0.96^{**}$). It can be concluded that with increasing biological yield, sepal yield will be increased. Biological yield and harvest index are important phenotypic traits that can be considered in breeding programs. So that, simultaneous increasing of biological yield and harvest index can be increased the performance of sepals.

Acknowledgment

We wish to thank Vice President for Research and Technology, University of Birjand, Iran for the financial support of a part of the project.

References

- Acquaah, G., Adams, M.W., Kelly, J.D., 1992. A factor analysis of plant variables associated with architecture and seed sizes in dry bean. Euphytica 60 (3), 171–177.
- Allah-Gholipour, M., 1997. Study of Correlation of Some Important Agronomic Traits with Rice Grain Yield by Path Analysis. (M.Sc. thesis) Tehran University, Iran. Babatunde, F.E., Mofoke, A.L.E., 2006. Performance of Roselle (*Hibiscus sabdariffa* L.) as
- influenced by irrigation schedules. Pakistan Journal of Nutrition 5 (4), 363–367.
- Bardideh, K., Kahrizi, D., Ghobadi, M.E., 2013. Character association and path analysis of black cumin (*Nigella sativa* L.) genotypes under different irrigation regimes. Notulae Scientia Biologicae 5 (1), 104–108.
- Chitra, R., Rajamani, K., 2010. Character association and path analysis in glory lily (Gloriosa superba L.). Communications in Biometry and Crop Science 5 (2), 78–82.
- Da-Costa-Rocha, L., Bonnlaender, B., Sievers, H., Pischel, I., Heinrich, M., 2014. Hibiscus sabdariffa L. – a phytochemical and pharmacological review. Food Chemistry 165, 424–443.
- Dalkani, M., Darvishzadeh, R., Hassani, A., 2011. Correlation and sequential path analysis in ajowan (*Carum copticum* L.). Journal of Agricultural Sciences 5 (2), 211–216.
- El-Boraie, F.M., Gaber, A.M., Abdel-Rahman, G., 2009. Optimizing irrigation schedule to maximize water use efficiency of *Hibiscus sabdariffa* under Shalatien conditions. World Journal of Agricultural Sciences 5 (4), 504–514.
- Fallahi, H.R., Ghorbany, M., Aghhavani-Shajari, M., Samadzadeh, A., Asadian, A.H., 2017a. Qualitative response of roselle to planting methods, humic acid application, mycorrhizal inoculation and irrigation management. Journal of Crop Improvement 31.
- Fallahi, H.R., Ghorbany, M., Aghhavani-Shajari, M., Asadian, A.H., Samadzadeh, A., 2017b. Influence of arbuscular mycorrhizal inoculation and humic acid application on growth and yield of roselle (*Hibiscus sabdariffa* L.) and its mycorrhizal colonization index under deficit irrigation. International Journal of Horticultural Science and Technology (in press).
- Fasoyiro, S.B., Babalola, S.O., Owosibo, T., 2005a. Chemical composition and sensory quality of fruit-flavoured roselle (*Hibiscus sabdariffa*) drinks. World Journal of Agricultural Science 1 (2), 161–164.
- Fasoyiro, S.B., Babalola, S.O., Owosibo, T., 2005b. Chemical and storability of fruit-flavoured (*Hibiscus sabdariffa*) drinks. World Journal of Agricultural Sciences 1 (2), 165–168.
- Futuless, K.N., Kwaga, Y.M., Clement, T., 2010. Effect of sowing date on calyx yield and yield components of rosselle (*Hibiscus Sabdariffa* L.) in northern Guinea savanna. New York Science Journal 3 (11), 1–4.
- Garcia de Moral, L.F., Rharrabti, Y., Elhani, S., Martos, V., Royo, C., 2005. Yield formation in Mediterranean durum wheat under two contrasting water regimes based on pathcoefficient analysis. Euphytica 146, 203–212.
- Hirunpanich, V., Utaipat, A., Morales, N.P., Bunyapraphatsara, N., Sato, H., Herunsalee, A., 2005. Antioxidant effects of aqueous extracts from dried calyx of *Hibiscus sabdariffa* Linn, (Roselle) in vitro using rat low-density lipoprotein (LDL). Biological & Pharmaceutical Bulletin 28 (3): 481–548.
- Horwitz, W., 1980. Official Methods for Analysis, 13th ed. Association of Official Analytical Chemists (AOAC), Washington, USA.
- Ibrahim, M.M., Hussein, R.M., 2006. Variability, heritability and genetic advance in some genotype of roselle (*Hibiscus Sabdariffa* L.). World Journal of Agricultural Sciences 2 (3), 340–345.
- Ibrahim, E.B., Abdalla, A.W.H., Ibrahim, E.A., El-Naim, A.M., 2013a. Interrelationships between yield and it components in some roselle (*Hibiscus sabdariffa* L.) genotypes. World Journal of Agricultural Research 1 (6), 114–118.
- Ibrahim, E.B., Abdalla, A.W.H., Ibrahim, E.A., El-Nai, A.M., 2013b. Variability in some roselle (*Hibiscus sabdariffa* L.) genotypes for yield and its attributes. International Journal of Agriculture and Forestry 3 (7), 261–266.
- Karuppaiah, P., Senthil Kumar, P., 2010. Correlation and path analysis in African marigold (*Tagetes erecta* L.). Electronic Journal of Plant Breeding 1, 217–220.
- Mehrabadi, H.R., Nezami, A., Kafi, M., Ramezani-Moghaddam, M.R., 2015. Yield, yield components, correlation coefficients and path analysis of cotton cultivars under drought stress. Journal of Crop Production and Processing 17, 217–227.
- Minitab Institute, 2014. Minitab Statistical Software, version 17.0, State College, PA 16801-3008.
- Nasri, R., Paknejad, F., Sadeghi-Shoa, M., Ghorbani, S., Fatemi, Z., 2012. Correlation and path analysis of drought stress on yield and yield components of barley (*Hordeum* vulgare) in Karaj region. Agronomy and Plant Breeding 8 (4), 155–165.
- Nemati-Lafmejani, Z., Tabaei, S.R., Lebaschi, M.H., Jaffari, A.A., Najafi-Ashtiani, A., Daneshkhah, M., 2011. Path analysis of *Rosa damascena* Mill. performance under different conditions. Iranian Journal of Medicinal and Aromatic Plants 27 (4), 561–572.
- Rafeie, F., Saeidi, G.A., 2005. Phenotypic and genotypic relationships between agronomic traits and yield components of Safflower. Scientific Journal of Agriculture 28, 137–147.
- Rahbarian, P., Afsharmanseh, G., Modafea Behzadi, N., 2011. Effect of drought stress as water deficit and planting density on yield of Roselle (*Hibiscus sabdariffa*) in Jiroft region. New Finding in Agriculture 5 (3), 249–257.
- Rezaei, A.M., Soltani, A., 1998. Introduction to Applied Regression Analysis, 1st ed. Isfahan University of Technology Press, Isfahan, Iran.
- Sabiel, S.A.I., Ismail, M.I., Osman, K.A., Sun, D., 2014. Genetic variability for yield and related attributes of roselle (*Hibiscus sabdariffa* L.) genotypes under rain-fed conditions in a semi-arid zone of Sudan. Persian Gulf Crop Protection 3 (1), 33–40.
- Sanjari-Mijani, M., Sirousmehr, A., Fakheri, B.A., 2015. The effects of drought stress and humic acid on some physiological characteristics of roselle (*Hibiscus sabdariffa*). Agricultural Crop Improvement 17 (2), 403–414.

- SAS Institute, 2002. The SAS System for Windows. Release 9.0. SAS Inst., Cary, NC, USA. Satyanarayana, N.H., Mukherjee, S., Roy, S., Priya, B., Sardar, K.K., Bandhopadhyay, P., 2015. Genetic divergence studies for fibre yield traits in Roselle (*Hibiscus sabdariffa*)
- L.) In terai zone of West Bengal. Journal of Crop and Weed 11, 90–94.
 Seiler, G.J., Stafford, R.E., 1985. Factor analysis of components of yield in guar. Crop Science 25 (6), 905–908.
- Sembok, W.W., Abu-Kassim, N., Hamzah, Y., Rahman, Z.A., 2015. Effect of mycorrhizal inoculation on growth and quality of roselle (*Hibiscus sabdariffa* L.) grown in soilless culture system. Malaysian Journal of Applied Biology 44 (1), 57–62.
- Shirzad, H., Ghorbany, M., 2015. Survey of arbuscular mycorrhizal fungi symbiosis with cotton root in north Khorasan province. Iranian Journal of Cotton Research 2 (2), 1–12.

Singh, S., Lal, R.K., Gupta, A.K., Sarkar, S., Chandra, R., Gupta, P., Lahiri, R., Rai, S.,

2015. Genetic variability, associations, and path analysis among most economic traits in red sorrel (*Hibiscus sabdariffa* L.). Journal of Herbs, Spices & Medicinal Plants 21 (2), 173–181.

- Sonar, B.A., Kamble, V.R., Chavan, P.D., 2013. Native AM fungal colonization in three *Hibiscus* species under NaCl induced salinity. Journal of Pharmacy and Biological Science 5 (6), 7–13.
- Swain, T., 1965. Analytical methods for flavonoids. In: Goodwin, T.W. (Ed.), Chemistry and Biochemistry of Plant Pigments. Academic Press, London, pp. 543–544.
- Tousi-Mojarad, M., Bihamta, M.R., 2007. Investigating grain yield and related quantitative characters of wheat using factor analysis. Journal of Agricultural Science 17, 97–107.
- Wright, S., 1921. Correlation and causation. Part 1: method of path coefficients. Journal of Agricultural Research 20, 557–585.