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FUNCTIONAL FACTOR ANALYSIS IN SESAME UNDER WATER - LIMITING STRESS: NEW CONCEPT ON AN OLD METHOD

ABSTRACT

Multivariate statistical analysis, through their ability to extract hidden relationship between various traits, has a wide application in breeding programs. Having physiological concept on the multivariate analysis, factor analysis was used to extract differential relationships between different components involving in assimilate partitioning in sesame under regular irrigation regime and limited irrigation. The analysis revealed that under regular irrigation regime, the stored and/or currently produced assimilates are allocated to the filling seeds. However, incidence of water shortage in the beginning of flowering time make shifts in assimilate partitioning from formation of new seeds or capsules to the not-matured pre-formed seeds, which results in seeds with more nutrient storage. This indicates the requirement for change in breeding strategies under sub-optimal condition. The possible common language between factor concept in multivariate analysis, QTLs in genetics, and transcription factors in molecular biology is indicated.

Key words: assimilate partitioning, factor analysis, QTL, sesame, transcription factor

INTRODUCTION

Breeding programs are aimed to develop new varieties with superior per-formance compared to the commercial varieties. This is gained through precise evaluation of newly emerged lines or hybrids in diverse conditions. Multivariate analysis approaches are widely applied to explore trends in the data obtained in

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the evaluation experiments. In this way, most of the efforts have been concentrated on elucidating relationships between the yield and its components. (Lee and Kaltsikes, 1973; Sabouri *et al.*, 2008; Salehi *et al.*, 2008; Mostafavi *et al.*, 2011; Al-Sayed *et al.*, 2012). However, most of the investigations were conducted under optimal growth condition. This could affect performance of the lines/hybrids when they are cultivated under sub-optimal conditions.

Plants are sessile organism, amenable to move when facing sub-optimal conditions. When meet environmental sub-optimal conditions, a plenty of modifications and alterations from molecular to whole plant level are triggered in the plants (Hodge *et al.*, 2009; Reisen *et al.*, 2005). Evolutionary speaking, many of the changes have been fixed in plant populations during long periods of stress occurrences (Shah *et al.*, 2011). The fixed modifications are defined under the criterion known as tolerance. Plant architecture, which is influenced by a combination genetic, developmental, and environmental cues are thought to be among the modifications (Doust, 2007; Zhang *et al.*, 2009).

Sesame (*Sesamum indicum* L.) is believed to be the oldest oilseed plant cultivated by human (Joshi, 1961). Sesame seed contains around 50% high quality oil containing large amounts of natural antioxidants. This crop is widely cultivated in arid and semi arid area (Witcombe *et al.*, 2007), and thus, is considered to be a drought tolerant crop (Boureima *et al.*, 2011).

Yied, as the final goal of agriculture, is defined as accumulation and partitioning of assimilates. These processes require energy capturing and circulating, in the form of carbohydrates, throughout the plant body. The circulation, which is defined as assimilate partitioning, is widely changed upon developmental and environmental conditions (Kasperbauer, 1987; Chaves, 1991; Stitt and Schulze, 1994, Geiger *et al.*, 1996; Chen & Reynolds, 1997; Taize and Zaiger, 1998). Molecular aspect of the assimilate partitioning in plants has been investigated (for example Geiger and Koch, 1996; Sauer, 2007). The complex nature of plant yield has been dissected into several influencing pathways, and, for many plants, prediction of plant behaviors under sub-optimal growth conditions is being applicable.However, because a plenty of known and un-known agents imply their effects on the physiology of plant yield formation, *e. g.*, assimilate partitioning, developing valid models to explain formation of the yield components needs to walk on multidimensional spaces. Each biotic and abiotic agent which affect, directly or indirectly, on the biology of plant constitute a space.

Multivariate analysis has been extensively used to discover the relationship between various yiled components (Lee and Kaltsikes 1973; Sabouri *et al.*, 2008; Salehi *et al.*, 2008; Mostafavi *et al.*, 2011; Al-Sayed *et al.*, 2012). For decades, mendelian genetic-based method in combination with the multivariate methods have been applied to improve varieties with high yield under optimal and sub-optimal conditions. These efforts hve been mirrored in releasing many high yield varieties (Araus *et al.*, 2008). Instead of great successes have been gained to date in releasing new verities, crops are reaching their yield potential

capacities, thus, the trend of increasing yield per crop is being plateau. (Sharma-Natu and Ghildiyal, 2005; Sinclair and Rufty, 2012). Unlike the plateau situation, there are increasing number of publications representing wide use of multivariate analysis methods. They usually do not consider biological concepts on the data. Integrating plant physiology to plant breeding can break the plateau dam and open new avenues in breeding programs.

In this paper, we try to come closer to the assimilate partitioning strategies undertaken by sesame when water shortage was implied in the field. Although conducting of the experiment was in the format of usual crop physiology researches and factor analysis, a multivariate approach, was used for data analysis, we were able to discover physiological relations contributing in response of sesame plant to drought stress and propose hypothesis for molecular investigations in future.

MATERIAL AND METHODS

Plant Materials

Twenty eight sesame F_3 families derived from 28 individual F_2 plants were grown and evaluated during spring and summer of 2011. The F_2 plants had been previously derived from a half diallel cross with seven parents.

Experiment Setting and Treatment

The experiment was conducted for normal irrigation condition and water limited regime, in two separate arrangements. Genotypes were arranged in a complete randomized block with three replications and this arrangement repeated once more in around 20 meters apart. In each arrangement, four lines of five meters in length were served as plots. The inter- and intra plant spacing were set to 60 and 10 cm, respectively. All agronomic activities including fertilizer application and weed control were performed in the usual way for both arrangements. One arrangement was irrigated normally up until harvest time. For the other arrangement, irrigation practices were stopped once the first flower was observed on plants.

Trait Evaluation and Measurements

For each population in each plot, five un-branched plants were considered for recording the data. Days to flowering (X_1) was recorded as days from emergence in the field to the appearance of the first flower. The time between the emergences of the first flower till the end of flowering period on the plants was recorded as flowering period (X_2). The time interval between seedling emergence and maturity of the first capsule was considered as the date for first capsule maturity (X_3). Total number

of capsule on the plant was counted and reported as the number of capsules per main stem (X_4). Height of the first capsule (X_5) was defined as the distance of the first node bearing capsule to the soil surface. The length of the main stem bearing capsule was considered as fertile length of main stem (X_6). To this end, the first capsule height was subtracted from whole plant height. Stem diameter (X_7) was measured in the middle of plant height by caliper. Weight of a random 100 seeds from each plant was measured and recorded as 100-seed weight (X_8). Length of five random capsules from each plant was measured and averaged to represent the capsule length (X_9). The distance between two successive nodes in the middle of plant height was considered as inter-node length (X_{10}). The number of nodes with or without capsule was recorded as the number of fertile nodes (X_{11}) and number of infertile nodes (X_{12}). Number of seeds in the five capsules used for measuring X_9 was counted and averaged over five to serves as the number of seed per capsule (X_{13}). Weight of whole seeds from individual plant was determined and cared as seed yield per plant (Y).

Data Analysis

Principal component analysis was performed based on correlation matrix and by MINITAB software ver11 for windows. Factor analysis was done using SPPS software ver 17 using principal component algorithm. The Kaiser varimax (pointed in (Fruchter 1967) was applied for extracting the factors.

RESULTS

Two separate experiments were run employing regular irrigation and stopping the regular watering at the beginning of flowering; thus for, two separate data sets were collected and analyzed.

As a method of choice in selecting the way of factor extraction, principal component analysis was used to get an idea about the maximum factors can be logically extracted. In this method, the number of factors to be extracted was defined based on behavior of eigen values against the component numbers.

To this end, the component number immediately after a steep reduction in eigen value trend was considered as the maximum number of factors to be extracted. The trends for both data sets are shown in Figures 1 and 2.

Predefinition of maximum number of factors based on the mentioned behavior of eigen values is somehow subjective. Another way in factor extraction relies on extracting factors whose eigen values are greater than 1. In the following, the results of factor analysis taking advantage of both above mentioned ways are presented. After extracting the factors, within each factor, traits with loading coefficient greater than 0.5 or less than -0.5 are considered to be influenced significantly by the respected factor (Fruchter1967). In this paper, the significant traits within each factor are called factor groups.
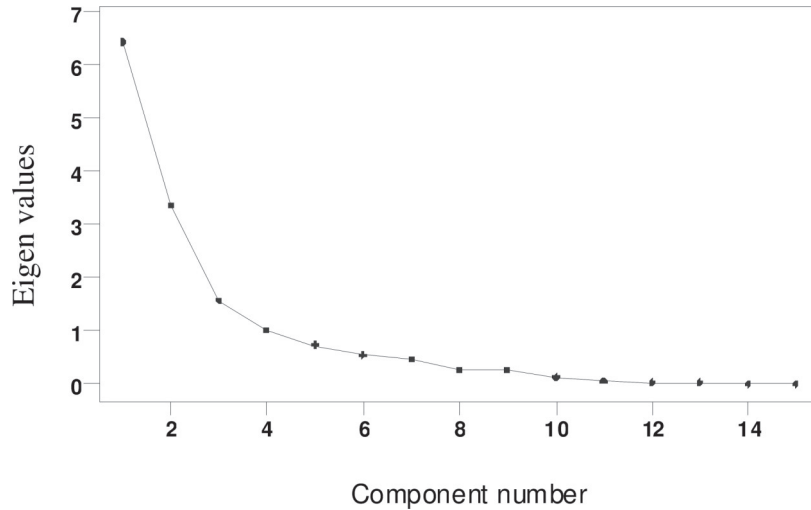
Fig. 1. Trend of eigen values over the components extracted from the data set for regular irrigation in sesame

Regular Irrigation Condition

Extracted factors for dataset of regular irrigation in sesame. Number of factors were pre-defined before the extraction. The data are presented here after varimax rotation with Kaiser normalization

Variable name	Factor 1	Factor 2	Factor 3
Days to flowering (X_1)	0.369	**0.759**[*]	-0.127
Flowering period (X_2)	**0.754**	0.179	-0.012
Days to first capsule maturity (X_3)	0.113	**0.887**	-0.019
Number of capsule per main stem (X_4)	**0.876**	0.072	-0.384
First capsule height (X_5)	0.333	**0.844**	0.263
Fertile length of main stem (X_6)	0.294	-0.130	**0.837**
Stem diameter (X_7)	**0.787**	0.413	0.227
100-seed weight (X_8)	-0.097	**0.779**	0.317
Capsule length (X_9)	-0.264	0.336	**0.773**
Inter-node length (X_{10})	0.364	0.346	0.250
Number of infertile nodes (X_{11})	0.437	**0.676**	0.275
Number of fertile nodes (X_{12})	**0.923**	0.123	0.227
Number of seed per capsule (X_{13})	-0.191	0.403	**0.706**
Seed yield per plant (Y)	**0.790**	0.090	-0.255

*: Bolded data highlight the absolute values greater than absolute value of 0.5

The pattern of changes in eigen value for various component is shown in Fig. 1. A steep reduction in eigen values was observed immediately before the third component. Thus for, the factor extraction algorithm was set to extract three factors (Table 1.(Extraction of factors based on eigen values greater than 1 led to extraction of four factors (Table 2). Results of the two ways of extraction were compared in Fig. 2. Yield- related traits in plants are connected to each other in a complex fashion. Many of them, on one hand, may have cause and effect relations, but on the other hand, they by themselves may be influenced by unknown factors. Thus for, having a simple view on the data and neglecting the complex inter- and intra- relationships between the traits is far away from the reality. Therefore, the results presented in Table 2 was thought to be more realistic.
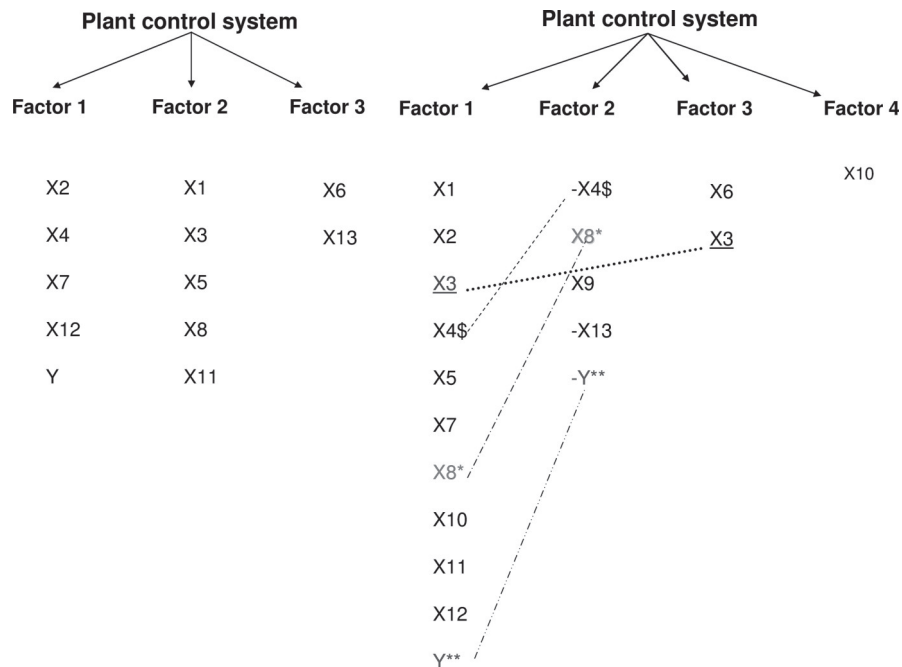

Fig. 2. Composition of factors extracted from data collected under regular irrigation regime. On the left scheme, the factors were extracted by pre-defining the factor numbers. The right scheme shows factor composition for the same data but with different algorithm. Here the extraction algorithm is set to extract factors with respected eigen values greater than 1. In this scheme some traits are appeared in more than one factor groups. These traits are specifically annotated with star (*), double stars (**), $, underlines (X), and also connected with lines

According to the results presented in Table 2, the first factor had effects on many traits such as days to flowering, flowering period length, days to maturity of first capsule, number of capsule on the main stem, distance of appearing the

first capsule to the soil surface, stem diameter, weight of seeds, inter-node length, and number of infertile nodes.

Table 2

Extracted factors for dataset of regular irrigation in sesame. Number of factors was determined considering eigenvalues greater than 1. The data are presented here after varimax rotation with Kaiser normalization

Variable name	Factor 1	Factor 2	Factor 3	Factor 4
Days to flowering (X_1)	**0.745***	0.010	-0.417	0.187
Flowering period (X_2)	**0.641**	-0.411	0.141	-0.121
Days to first capsule maturity (X_3)	**0.680**	0.297	**-0.500**	0.032
Number of capsule per main stem (X_4)	**0.567**	**-0.773**	-0.027	-0.100
First capsule height (X_5)	**0.868**	0.319	-0.193	-0.153
Fertile length of main stem (X_6)	0.308	0.293	**0.790**	0.248
Stem diameter (X_7)	**0.880**	-0.178	0.189	0.112
100-seed weight (X_8)	**0.539**	**0.599**	-0.261	0.214
Capsule length (X_9)	0.225	**0.807**	0.279	-0.092
Inter-node length (X_{10})	**0.546**	0.076	0.102	**0.709**
Number of infertile nodes (X_{11})	**0.828**	0.189	-0.050	-0.319
Number of fertile nodes (X_{12})	**0.776**	-0.390	0.406	-0.133
Number of seed per capsule (X_{13})	0.306	**0.747**	0.215	-0.411
Seed yield per plant (Y)	**0.550**	**-0.627**	0.027	-0.104

*: Bolded data highlight the absolute values greater than absolute value of 0.5

Limited Irrigation Condition

According to the behavior of the eigen values over the component numbers, which showed a steep reduction in the eigen values immediately before the third component (Fig. 3), the extraction algorithm was set to extract three components. Interestingly, running the algorithm for factor extraction based on eigen values greater than 1 led to the same results, which are depicted in Table 3.Two phenological related traits, that is, days to starting flowering and days to starting maturity along with 100-seed weight, and number of infertile nodes were co-coordinately affected by Factor 1. Meanwhile, this factor affected, in the opposite direction, on fertile length of main stem. Factor 2 affects on flowering period, number of capsule per main stem, stem diameter, number of fertile nodes, and seed yield. Regardless of fertile length of main stem, capsule length and number of seed per capsule are affected by a single Factor, Factor 3.
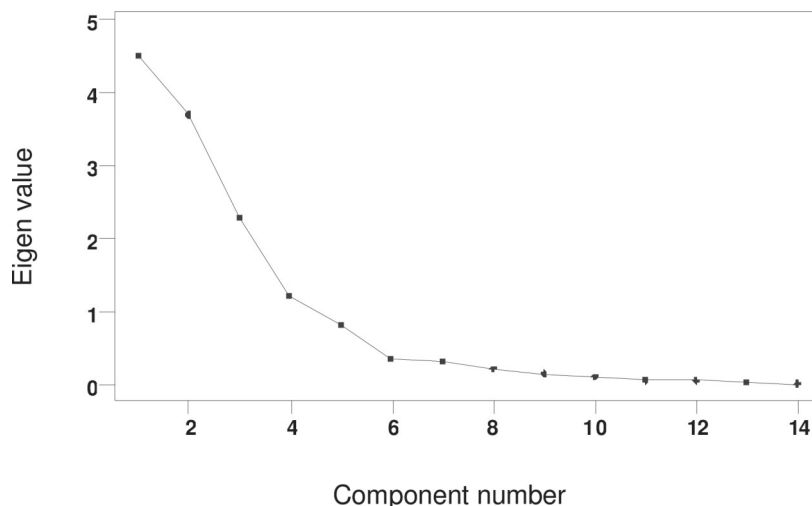
Fig. 3. Trend of eigen values over the components extracted from the data
set for limited irrigation condition in sesame

Factor 4 strongly inserts its effect on internodes length, but on the opposite direction affects on flowering period. Flowering period affects on capsule formation through number of capsule per plant (Masouri and Najafabadi, 2004).

Table 3

Extracted factors for dataset of limited irrigation condition in sesame. Number of Factors was determined considering eigen values greater than 1. The data are presented here after varimax rotation with Kaiser normalization

Variable name	Factor 1	Factor 2	Factor 3	Factor 4
Days to flowering (X_1)	**0.865***	0.032	0.219	0.240
Flowering period (X_2)	0.321	**0.567**	-0.343	**-0.545**
Days to first capsule maturity (X_3)	**0.912**	-0.021	0.172	0.035
Number of capsule per main stem (X_4)	0.001	**0.865**	-0.433	0.033
First capsule height (X_5)	**0.906**	0.129	-0.134	-0.186
Fertile length of main stem (X_6)	**-0.682**	0.162	**0.644**	-0.020
Stem diameter (X_7)	0.274	**0.665**	0.239	0.366
100-seed weight (X_8)	**0.692**	-0.253	0.487	0.115
Capsule length (X_9)	-0.007	-0.105	**0.913**	0.101
Inter-node length (X_{10})	0.036	0.013	-0.021	**0.815**
Number of infertile nodes (X_{11})	**0.841**	0.298	-0.313	-0.206
Number of fertile nodes (X_{12})	0.029	**0.831**	0.081	-0.391
Number of seed per capsule (X_{13})	0.137	-0.052	**0.809**	-0.027
Seed yield per plant (Y)	-0.119	**0.943**	-0.067	0.036

*: Bolded data highlight the absolute values greater than 0.5

DISCUSSION

Plants, as sessile organisms, have to manage their behavior under variable environmental conditions. Different behavior of plants can be surveyed through comparative analysis of plants employing various conditions. Multivariate analysis methods are widely used for extracting hidden facts among the data collected on biological systems (for example see Lucas *et al.*, 2010).

In this research we applied factor analysis to data obtained from sesame plants experienced water shortage from initiation of flowering onward. The normal water supply was used as a control. In factor analysis procedure, extraction of factors is executed in a way so that subsequence factors have less variance than the previous ones. Thus for, the traits affected by the first factor are expected to participate more in the total variation in the genotype population under investigation. It could be deduced that in the order number of factors, the earlier factors, and subsequently the included traits, show the main structure of the genotypes under investigation better than the later ones. Of course, the kind of the structure depends on the traits subjected to the analysis.

The different physiological structure of sesame plant under different environmental situation is prominent in the composition of the factors in both conditions. Sesame breeding programs, which are aimed toward gaining high seed yield, are being preceded in the research stations usually under the optimum condition. Thus, seed yield and its components are appeared as the most explanatory characters for the genotype structure. Theses traits are presented in the Factor 1 list (Table 2). We can suppose that a regulatory shunt (see Figure 2) simultaneously affects on many phonological traits such as times related to flowering and maturity and assimilate production and storage structures. This goes toward increasing the yield-related structures such as number of capsules and assimilates reservation room such as stem volume. The final goal here is producing more seed yield.

Formation of capsule, as the most prominent component of the seed yield (Mansouri & NaJafabadi, 2004) is influenced by the number of nodes bearing generative buds. On the other hand, leaves are formed on non-fertile nodes,, and produce assimilates which are exported to the forming capsules. Assimilates produced before formation of capsules are thought to be stored in the stem and translocated into the forming seeds (Narayanan & Reddy, 1982), a phenomenon known as re-mobilization. Stem diameter, number of total nodes (fertile and infertile), and inter-node define the stem dimension. These traits are determinants in providing assimilate to the seeds, and thus on seed weight (that is, 100-seed weight). In an invariant main stem length, increasing height of the first capsule from the soil surface reduces number of capsule per main stem; however, providing more infertile nodes which bear leaves, and thus more photosynthesis source.

Appearing of seed yield in the same factor group of the above mentioned traits indicates that assimilate storage and partitioning is controlled by at least one common factor. The high correlation between the seed yield and number of capsule per plant (Bamerjee & Kole, 2006) is highlighted one more time in the structure of Factor 2. In this table, the two traits appear to have negative loading factor values. It is interesting that number of capsule per main stem and seed yields, both, are influenced by Factor 1, too. This means that the control of yield via it's most important component is regulated in a two step procedure. The first step is governed via Factor 1 and the second step by making a balance in competition between the traits and some members of yield components such as seed per capsule and 100-seed weight. Capsule length, 100-seed weight, and number of seed per capsule are, together, influenced by a single factor. The differential and opposite effect of Factor 2 on the group traits "seed yield and number of capsule per main stem "versus" 100-seed weight, capsule length, and number of seed per capsule" indicate that the factor governs the competition in assimilate partitioning. The data in Table 2 reveal that the lengths of main stem bearing capsules and days to first capsule maturity are negatively affected by a factor, Factor 3. However, the time length to maturity of the first capsule is influenced by another factor, Factor 1. Thus, we can deduce that the physiological processes involved in starting maturity in sesame capsule could not be simply modeled, and a complex network is involved. Storage of assimilates serves as a very important sources in seed filling, specifically at the later stages of development. So it seems that these is a mechanism containing two levels of regulations to affect on the room for assimilate reservation, *e g.*, internode lengths. These two levels, are affecting either independent of other traits, merely via Factor 4, or in a combinatorial way with many other traits, through Factor 1.

Unlike the regular watering condition, the seed- and capsule-related characters, except for capsule number per main stem, were not appeared in the same group as seed yield. There are lines of evidence indicating that under optimum condition, seed yield in sesame is determined by number of capsule per plant, number of seed per capsule, and 100-seed weight (Mansouri and Soltani, 2004; Banerjee and Kole, 2006). Referring to our results under limiter irrigation, we can get the conclusion that deviating environmental condition from optimum make changes in yield structure of sesame plant. That is, a new set of traits are coordinately affected by the same factor as the seed yield is.

In contrast to the results gained under the regular irrigation condition, fertile length of the main stem is not controlled by a single factor, but rather is regulated by two factors, Factors1 and 3.As the fertile nodes bear capsule and leaf, this trait regulates number of leaves (source) and capsule (sink), thus is very important in making a balance between sink and source. We may get the idea that under water limitation, the plant uses different pathway of regulations (that is via two separate Factors) on controlling the balance between sink and source.

Capsules are strong sinks, thus, lack of severe control on the generation of the strong sink triggers strong competition in the plant body which reduce the viability of the plant under water limiting condition (Blum, 1996). The different behavior of factor 4 extracted under water shortage condition on flowering period and internodes length could emphasize that in the stress situation, plant tries to manage allocation of assimilates to filling the pre-formed seeds through reducing formation of new capsule in one hand, and, on the other hand, increase spaces for reserving assimilate (internodes length) for filling the grains when the stress severity increases during the time course. So, it is not excluded that-based on the experienced situation, plant can predict the future (Xu *et al.*, 2010) and fortify itself to allocate more assimilate to the pre-prepared grain for surviving. The direct mirror of the phenomenon could be increasing the seed weight. This assumption is partly supported when under the limited water condition, the100-seed weight is appeared in the list of traits controlling by Factor 1.

Under sub-optimal condition, such as water limiting, however, the decision center of plant shift the strategy of producing more seed yield toward ecological surviving. This is gained through producing seeds with more fitness. Ecologically and evolutionary speaking, seeds with more nutrition reservation can survive in the nature and thus have more fitness. Therefore, under limited water regime, the prominent physiological structure of plant, Factor 1, includes 100-seed weight. The concept of the decision center becomes more highlighted when Factor 1 suppressed production of more capsules on the stem. This idea is presented based on the negative effect of the factor on fertile length of main stem and its positive effect on the first capsule height (Table 3).

The results mentioned above make a challenge on the stress breeding strategies. Selecting for varieties with high yield under optimum condition has been presented as an efficient strategy in stress breeding (Rosielle & Hamblin, 1981). However, the physiologic structure of sesame plants under limited water availability indicates that the plant invest the energy and assimilate for increasing the fitness of the progenies rather than producing more seeds. Accordingly, the high yielded varieties which have been identified in evaluating experiments under optimum condition are not expected to produce anaccepptable yield when experiencing water shortage situation.

Quantitative trait loci (QTLs) are regions or segments of chromosomes with high effects on quantitative traits. All the traits discussed in this paper have continues distributions and, thus, are considered as quantitative traits. As the quantitative traits are controlled by more than one QTL, we come to the point that the representation of traits within different factor compositions might indicate that the factors serve as QTLs. On the other hand, emerging the concept of pleiotropic QTLs (Mangin *et al.*, 1998; Lawson *et al.*, 2011) may explain effects of each factor as QTL on different traits.

Knowing the architecture and profile of plant assimilate partitioning is critically required to plant molecular breeders and physiologists. Here, although, we

are dealing with data obtained on whole plant measurements,it is taken into account that the biological processes in the whole plant scale are built up of small scale molecular events (Chaves *et al.*, 2003). Lines of evidences prove that instead of chromosomal segment, sometimes, QTLs can be defined as cis-regulatory elements that control expression level of downstream genes (Brem *et al.*, 2002; Schadt, *et al.*, 2003; Gilad *et al.*, 2008). So, the factors in the multivariate analysis might be considered to have the concept of QTLs or transcription factors (TFs) in molecular biology. Transcription factors are master molecule which recognize and bind to regulatory *cis*-elements in genome and thereby regulate expression of genes and so control many biological activities. Also having binding sites on promoters of many genes, each TFmayaffects on many downstream genes, a phenomenon led them to play as cross-talk between different pathways.

The validity of the linking between factor concept in the multivariate analysis and of QTLs in genetics and TFs in molecular biology remains to be a big open question, and need to be precisely investigated through detailed molecular experiments. Nevertheless, taking in mind the common language in the factor space, will directs the molecular plant biologists to accurately work on, or engineer a series of traits which are under control of a Factor, instead of a single trait.

CONCLUSION

When analyzing yiled and yiled-related characters in plants, a combination of mulativata analysis with systems biology view has to be applied. In this way, the meaningless output of the statistical softwares is implemented in a biological contex. As results, molecular interprations can be gained even when no real molecular experiments are performing.

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