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Assess Correlation, Stepwise Regression and Path Coefficient Analysis of Characteristics Affecting Seed Yield of Canola (*Brassica napus* L.) Genotypes

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ABSTRACT

BACKGROUND: Correlation and path coefficient analysis could be used as an important tool to evaluate relation between traits, propose most effective trait on seed yield and determine direct and indirect effects of selected traits on dependent variable.

OBJECTIVES: Evaluate correlation between traits and selected effective traits those had the highest effect on seed yield by stepwise regression and determine direct and indirect effect of selected traits on seed yield by path analysis.

METHODS: This research was done via combined analysis split plot factorial experiment based on randomized complete blocks design (RCBD) with three replications along 2015-16 and 2016-17. The main factor included different level of canola genotype (Hyola401, RGS003, Jerry) and sub factors consisted different concentration of gibberellin hormone (0, 50 and 100 mg.l^{-1}) and different times of application of gibberellin hormone (Planting, vegetative phase before flowering, flowering until pod emergence).

RESULT: Correlation between traits revealed the most positive and significant relation between biologic yield (r=0.960**), number of pod per plant (r=0.931**), number of seed per pod (r=0.905**), 1000-seed weight (r= 0.834**), pod length (r= 0.824**), harvest index (r= 0.690**) and seed yield was observed. Also the traits of leaf dry weight (r=0.550*), stem dry weight (r=0.530*) and plant height (r=0.510*) had significant correlation with the seed yield at 5 percent probability level. Stepwise regression analysis introduced five selected traits (biologic yield, number of pod per plant, number of seed per pod, 1000-seed weight and pod length determines) that covered 93.5 percent of variation related to seed yield. Variable of the biological yield with a positive direct effect (0.785) on seed yield with indirect effects of number of pods per plant (0.130), number of seed per pods (0.025), 1000-seed weight (-0.030) and pod length (0.05) caused a positive correlation between this trait and seed yield. So breeders can use selected traits for achieve optimum genotypes instead of direct selection for seed yield.

CONCLUSION: Among studied traits, biologic yield had the most direct positive effect (0.785) on seed yield and had an important role to explain seed yield variation.

KEYWORDS: *Gibberellin, Morphology, Nitrogen, Oil, Protein, Rapeseed.*

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1. BACKGROUND

Seed yield, as the most important quantitative characteristic, will be a result of genotype, environment and genotype-environment interaction effects (Marjanovic-Jeromela *et al*., 2009). Improvement of seed yield in canola has been the main objective of canola breeders for many years (Mahasi and Kamundia, 2007). Seed yield is a quantitative trait, which is principally influenced by the environment and consequently has a low heritability (Ana *et al*., 2009). Average yield of Canola can be improved up to its genetic potential which is much higher than the present status. Direct and indirect selection via yield components provides the basis for its successful breeding program and hence the yield-complexity problem can be more effectively tackled. Use of simple correlation analysis could not fully explain the relationships among the traits. Therefore, the path coefficient analysis has been used by many researchers for a more complete determination of the impact of independent variables on dependent one (Ali *et al*., 2003). In plant breeding factor analysis is mainly applied as structure detection method, and sometimes it can be used as index selection for improving more than one trait. Factor analysis was used to determine structural factors related to growth trait and yield components and also to detect factors relating to environmental stress including drought resistance in B. napus (Naderi and Emam, 2010). The main applications of factor analytic techniques are to reduce the number of variables and to detect structure in the relationship between variables (Sharma, 1996). Determination of correlation coefficients is an important statistical procedure to evaluate breeding programs for high yield as well as to examine direct and indirect contributions to yield variables (Semahegn Belete, 2011). Correlation analysis helps researchers to discriminate a significant relationship between traits. Non-important traits in the regression model will be omitted via a stepwise regression analysis, therefore the most important traits which have considerable effects on the dependent variable will be verified. Most of the traits which were selected through the stepwise regression model can be used as selection criteria for indirect selection in a breeding program (Sabaghnia *et al*., 2010). The path coefficient analysis helps the breeders to explain direct and indirect effects and hence has extensively been used in breeding programs in different crop species by various researchers (Ali *et al*., 2002). Stepwise multiple regression analysis is used to determine the contribution of more important traits that have significant association with seed yield (Golkar *et al*., 2011). As a result, the response to direct selection for seed yield may be unpredictable, unless there is good control of environmental variation. Plant breeders are seldom interested in a single trait and therefore, there is the need to examine the relationships among different traits, especially between seed yield and other traits. As the number of independent variables influencing a particular dependent variable increases, a certain amount of interdependence is expected. In such situations, correlations may be inadequate to explain the associations in a way that will enable breeders to decide on a direct or indirect selection strategy (Ofori, 1996). Hashemi *et al*. (2008) by assessment of relationship among seed yield and different traits reported correlation coefficient analysis of seed yield showed positive and significant relationship between selected traits with traits days to shooting, days from planting to full flowering, plant height, and number of seed per pod, 1000 seed weight, biological yield, harvest index, seed oil percent and oil yield. Step-wise regression of seed yield as dependent variable and the other traits as independent variables revealed that 98.9% of variation exists in seed yield accounted for by the traits biological yield, harvest index, days to flowering initiation and number of seed per pod. Path analysis for seed yield based on traits that correlated positively and significantly with this trait designed high efficiency of traits 1000 seeds weight, biological yield, harvest index, days to shooting and number of seed per pod as indirect selection criteria for genetic improvement of this trait in canola cultivars especially in early generations of breeding programs. The use of genotypic correlation helps evaluating the magnitude and direction of associations between characters facilitating the application of indirect selection, because genetic changes in a given trait may change other traits, leading to faster and larger genetic gains in plant breeding programs. Therefore, the selection for another trait may result in indirect response in the low heritable trait, provided the following conditions are satisfied: the genetic correlation between them is substantial, and the heritability of the secondary trait is greater than that of the primary trait (Ismaili *et al*., 2017).

2. OBJECTIVES

Current study was done to evaluate (i) the associations between yield components and other crop traits with seed yield, (ii) to determine the direct and indirect effects of the yield components and the crop traits on seed yield (iii) to discuss the interrelationships among the examined traits, (iiii) to provide the theoretical foundations to guide canola breeders who are researching the genetic correlation of the main agronomic characters and their influence on canola productivity under studied condition.

3. MATERIALS AND METHODS

3.1. Field and Treatments Information

This research was carried out to evaluate effect of different concentration and time of application of gibberellin hormone on canola genotypes production via combined analysis split plot factorial experiment based on randomized complete blocks design with three replications along two agronomic years (2015-16 and 2016-17). Place of research was located in Ahvaz city at longitude 48°40'E and latitude 31°20'N in Khuzestan province (Southwest of Iran). The main factor included different canola genotype (Hyola401, RGS003, Jerry) and sub factors consisted different concentration of gibberellin hormone (0, 50 and 100 mg.l^{-1}) and different time of application of gibberellin hormone (Planting, vegetative phase before flowering, flowering until pod emergence). This experiment had 27 plots. Each plot consisted of 8 lines with a distance of 30 cm and 5 meters length. The distance between the shrubs on every row was 5 cm.

3.2. Farm Management

Base fertilizers (50 kg.ha^{-1} Nitrogen from urea, 100 kg.ha^{-1} phosphorus from ammonium phosphate and 100 kg.ha^{-1} potassium from potassium sulfate) were added to the soil based on soil tests and the recommendations of the Iranian Soil and Water Research Institute at the planting stage. Also 100 kg.ha^{-1} Nitrogen was added to the soil at stem elongation phase. The light-disk harrow was used to mix the soil and the fertilizer after soil fertilization. The furrows were covered with soil. The seeds were planted 3 cm above the fertilizer. Physical and chemical properties of the soil are mentioned in table 1. To apply the first stage of gibberellin hormone before planting the seeds were soaked in three concentrations of the hormone overnight. The second stage of gibberellin application was done in the vegetative growth stage before flowering. The last stage of gibberellin application was done during the flowering to pod emergence stage.

Table 1. Physical and chemical properties of studied field

Soil depth (cm)	**Acidity (pH)**	**Electrical conductivity (ds.m^{-1})**	**Organic carbon (%)**	**Absorbable Phosphorus (ppm)**	**Absorbable potassium (ppm)**
0-30	7.76	7.55	0.55	9.11	184
Clay (%)	**Silt (%)**	**Sand (%)**	**Soil texture**	**ρ_b (gr.cm^{-3})**	**Fe (ppm)**
33	37	30	Clay	1.29	10.4

3.3. Measured Traits

In order to determine the yield components during physiologic maturity, 10 plants were chosen randomly from each plot. Then 1000-seed weight, number of pod per plant and number of seed per pod were assessed. In final harvest area, one- square meter of each plot, seed yield were calculated. In addition, seed samples were dried, weighed and analyzed for oil content. Oil content determine by Near Infrared Spectroscopy (Sato, 2002). Results for oil content expressed on 8.5 % moisture. Oil yield calculated by multiplying seed yield by oil content. Kjeldahl method was used to determine the amount of plant nitrogen content. Finally, nitrogen percentage was calculated as follow (Sosulski and Imafidon, 1990): **Equ. 1.** Protein content= Nitrogen percentage×5.7. Protein yield calculated by multiplying seed yield by protein content. Harvest index (HI) was calculated according to formula of Gardener *et al*. (1985) as follows: **Equ.2.** HI= (Seed yield/Biologic yield) ×100.

3.4. Statistical Analysis

Analysis of variance was done via SAS (Ver.8) software. For studying the type of relations between the independent variables (agronomic traits and yield components), and the dependent variable (seed yield), seed yield path coefficient analysis was performed in order to achieve the direct and indirect effects. For doing statistical calculations, identifying correlation coefficients and regression analysis, Minitab (ver.15) software was utilized, and for coefficient analysis, Path74 software was used.

4. RESULT AND DISCUSSION

4.1. Correlation between traits

Result of analysis of variance was mentioned in table 2. Simple correlation coefficients between studied traits were estimated according to Pearson coefficient. Result revealed the most positive and significant correlation between biologic yield ($r=0.960^{**}$), number of pod per plant ($r=0.931^{**}$), number of seed per pod ($r=0.905^{**}$), 1000- grain weight ($r= 0.834^{**}$), Pod length ($r= 0.824^{**}$), harvest index ($r= 0.690^{**}$) and seed yield was observed. Also the traits of leaf dry weight ($r=0.550^{*}$), stem dry weight ($r=0.530^{*}$) and plant height ($r=0.510^{*}$) had significant correlation with the seed yield at 5 percent probability level (Table 3). Soltani Howyzeh *et al*. (2018) by compare seventhin spring canola reported the correlation coefficients among the seed yield and 1000-seed weight, number of seed per pod, harvest index and days to maturity were positive and significant. Results of stepwise regression analysis revealed that 1000-seed weight, number of pods per plant and days to maturity had significantly effects on seed yield. Path coefficient analysis revealed that the number of seed per pod and 1000-seed weight had the largest direct effects on grain yield, its seams possible to be use as selection criteria in breeding programs for improving grain yield of spring rapeseed cultivars. Due to the high correlation between biological yield and seed yield and the high effect of this variable on seed yield, the selection process can be done based on this important trait. In order to produce more genetic diversity for breeding, researchers crossed the genotypes with higher biological yield and used the superior genotypes had mentioned trait. The correlation between grain yield variable and all its components was positive and significant and showed the effect of the above traits on final yield (Table 3). Increased biological yield and its direct relationship with seed yield showed a relationship between photosynthetic efficiency and grain yield, so, that genotypes had more apply of production factors and accumulate more photosynthetic materials in their sink have more efficiency and yield. Mentioned result was similar to finding of Amor (2018). The increase of biologic yield and its direct relation with seed yield show the relations between photosynthesis efficiency of plant and seed yield, therefore genotypes which have gained more profit of production factor according to growth conditions and they keep more photosynthesis materials in their sinks, have more efficiency. This status was in conformity with the results of some other researchers (Mardin, 2017; Tian, 2017).

Table 2. Result analysis of variance of measured traits

S.O.V	df	Plant height	Number of side branches	Pod length	Stem dry weight	Leaf dry weight
Year	1	ns	ns	ns	ns	ns
Replication × Year	4	ns	ns	ns	ns	ns
Genotype (G)	2	*	**	*	*	**
G × Year	2	ns	ns	ns	ns	ns
Gibberellin Concentration (GC)	2	**	**	*	*	**
GC × Year	2	ns	ns	ns	ns	ns
G × GC	4	ns	ns	ns	ns	ns
Year × G × GC	4	ns	ns	ns	ns	ns
Time of Gibberellin application (TGA)	2	*	**	*	*	**
TGA × Year	2	ns	ns	ns	ns	ns
TGA × G	4	ns	ns	ns	ns	ns
TGA × G × Year	4	ns	ns	ns	ns	ns
GC × TGA	4	ns	ns	ns	ns	ns
Year × GC × TGA	4	ns	ns	ns	ns	ns
G × GC × TGA	8	ns	ns	ns	ns	ns
Year× G × GC × TGA	8	ns	ns	ns	ns	ns
CV (%)	-	9.27	6.81	8.84	5.37	7.50

ns, * and **: no significant, significant at 5% and 1% of probability level, respectively.

Continue of table 2.

S.O.V	df	No. pod per plant	No. seed per pod	1000-seed weight	Seed yield	Biologic yield	Harvest index
Year	1	ns	ns	ns	ns	ns	ns
Replication × Year	4	ns	ns	ns	ns	ns	ns
Genotype (G)	2	**	*	*	**	**	*
G × Year	2	ns	ns	ns	ns	ns	ns
Gibberellin Concentration (GC)	2	*	*	*	*	*	*
GC × Year	2	ns	ns	ns	ns	ns	ns
G × GC	4	ns	ns	ns	ns	ns	ns
Year × G × GC	4	ns	ns	ns	ns	ns	ns
Time of Gibberellin application (TGA)	2	*	*	*	*	*	*
TGA × Year	2	ns	ns	ns	ns	ns	ns
TGA × G	4	ns	ns	ns	ns	ns	ns
TGA × G × Year	4	ns	ns	ns	ns	ns	ns
GC × TGA	4	ns	ns	ns	ns	ns	ns
Year × GC × TGA	4	ns	ns	ns	ns	ns	ns
G × GC × TGA	8	ns	ns	ns	ns	ns	ns
Year× G × GC × TGA	8	ns	ns	ns	ns	ns	ns
CV (%)	-	9.52	8.53	5.13	6.32	7.68	8.60

ns, * and **: no significant, significant at 5% and 1% of probability level, respectively.

Continue of table 2.

S.O.V	df	Oil content	Oil yield	Protein content	Protein yield
Year	1	ns	ns	ns	ns
Replication × Year	4	ns	ns	ns	ns
Genotype (G)	2	*	*	*	**
G × Year	2	ns	ns	ns	ns
Gibberellin Concentration (GC)	2	**	*	*	*
GC × Year	2	ns	ns	ns	ns
G × GC	4	ns	ns	ns	ns
Year × G × GC	4	ns	ns	ns	ns
Time of Gibberellin application (TGA)	2	*	*	*	*
TGA × Year	2	ns	ns	ns	ns
TGA × G	4	ns	ns	ns	ns
TGA × G × Year	4	ns	ns	ns	ns
GC × TGA	4	ns	ns	ns	ns
Year × GC × TGA	4	ns	ns	ns	ns
G × GC × TGA	8	ns	ns	ns	ns
Year× G × GC × TGA	8	ns	ns	ns	ns
CV (%)	-	6.19	8.58	7.65	9.89

$^{ns,\ *\ and\ **}$: no significant, significant at 5% and 1% of probability level, respectively.

The significant and positive correlation between harvest index and seed yield indicate efficiency and kind of photosynthesis materials distribution in different parts of plant, especially in seed. Results of Xianc (2018) verify the mentioned issues. Due to remarkable effects of sowing dates on component characters and seed yield, the most important traits such as phenological and morphological traits, plant height and pods were significantly decreased at late planting dates and therefore seed yield was decreased (Siadat and Hemayati, 2009).

4.2. Stepwise regression analysis

In Stepwise regression analysis, seed yield was considered as a dependent variable, while other traits were considered as independent variables. All the studied traits were put into regression model and finally five traits of biologic yield, number of pods per plant, number of seed per pod, 1000-seed weight and pod length remained in the regression model. The mentioned model generally determines 93.5 percent of variation related to the seed yield (Table 4). Shahraki *et al*. (2013) by compare canola cultivar reported oil yield had significant positive correlation with seed yield, weight of 1000 seeds and oil content while had a negative correlation with number of days to flowering. Step wise regression showed that seed yield, oil content and days to flowering had the most effect on oil yield and justified 99.1% of regression model. The most direct effect on oil yield was belonging to oil content.

Table 3. Correlation between studied traits

Trait	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**
1	-													
2	0.51^{*}	-												
3	0.43^{ns}	0.15^{ns}	-											
4	0.75^{**}	0.89^{**}	0.72^{**}	-										
5	0.54^{*}	0.91^{**}	0.42^{ns}	0.82^{**}	-									
6	0.22^{ns}	0.59^{*}	0.34^{ns}	0.52^{*}	0.59^{*}	-								
7	0.81^{**}	0.79^{**}	0.56^{*}	0.59^{*}	0.84^{**}	0.95^{**}	-							
8	0.55^{*}	0.53^{*}	0.78^{**}	0.42^{ns}	0.51^{*}	0.69^{**}	0.96^{**}	-						
9	0.40^{ns}	0.41^{ns}	0.94^{**}	0.38^{ns}	0.25^{ns}	0.51^{*}	0.53^{*}	0.83^{**}	-					
10	0.35^{ns}	0.32^{ns}	0.96^{**}	0.31^{ns}	0.21^{ns}	0.52^{*}	0.73^{**}	0.90^{**}	-0.88^{**}	-				
11	0.37^{ns}	0.39^{ns}	-0.54^{*}	0.29^{ns}	0.22^{ns}	0.55^{*}	0.77^{**}	0.93^{**}	0.59^{*}	0.49^{ns}	-			
12	0.41^{ns}	0.16^{ns}	0.51^{*}	0.46^{ns}	0.18^{ns}	0.15^{ns}	0.25^{ns}	0.48^{ns}	0.33^{ns}	0.77^{**}	0.59^{*}	-		
13	0.45^{ns}	0.18^{ns}	0.53^{*}	0.45^{ns}	0.20^{ns}	0.13^{ns}	0.28^{ns}	0.43^{ns}	0.37^{ns}	0.75^{**}	0.61^{*}	0.97^{**}	-	
14	0.15^{ns}	0.25^{ns}	0.54^{*}	0.33^{ns}	0.24^{ns}	0.18^{ns}	0.32^{ns}	0.49^{ns}	0.39^{ns}	0.71^{**}	0.55^{*}	-0.66^{*}	-0.55^{*}	-
15	0.13^{ns}	0.23^{ns}	0.56^{*}	0.31^{ns}	0.23^{ns}	0.25^{ns}	0.24^{ns}	0.44^{ns}	0.31^{ns}	0.74^{**}	0.54^{*}	-0.76^{**}	-0.59^{*}	0.95^{**}

$^{ns,\ *\ and\ **}$: no significant, significant at 5% and 1% of probability level, respectively.

1: Leaf dry weight, **2**: Stem dry weight, **3**: Pod length, **4**: Number of side branches, **5**: Plant height, **6**: Harvest index, **7**: Biologic yield, **8**: Seed yield, **9**: 1000-seed weight, **10**: Number of seed per pod, **11**: Number of pod per plant, **12**: Protein yield, **13**: Protein content, **14**: Oil yield, **15**: Oil content.

Totally in breeding program for canola and based on stepwise results weight of 1000 seeds and seed yield are most important trait to selection canola cultivar for more oil content. Other traits which were studied did not have a significant influence on this model, therefore different varieties according to grain yield, are because of differences in the mentioned traits above. In a research project in stepwise regression analysis of traits of pod per plant verified 64% of coefficient, the number of grains per pod 67%, 1000- grain weight 72%, oil percentage78% and the number of nodes in stem verified 80% coefficient of changes in regression model, which were related to Canola varieties comparison (Baradaran *et al*., 2006). In regression model conducted by some researchers, in order to determine effective traits on bean yield, traits of 100 seed weight, total number of pods and the number of grains per pod were entered into the model (Rahnamaee tak *et al*., 2007). The results of stepwise regression analysis on bean varieties indicated that, 5 traits of pod weight, the number of grains in plant, the total number of pods, biological yield and harvest index justified 97% of the changes in grain yield, yet" pod weight" devoted 95% of changes to itself (Amini, 2009).

Table 4. The stepwise regression for yield and other traits as the dependent variable as independent variables

Variable added to model	The stepwise regression				
	1	2	3	4	5
Constant	-1499	-1855	-1765	-1721	-1839
Biologic yield	197	154	153.7	145.1	144.7
Number of pod per plant		1.02	1.16	0.97	0.75
Number of seed per pod			-2.1	-2.2	-4.1
1000-seed weight				111	141
Pod length					3.1
Coefficient R^2 (%)	44	64	73	86.6	93.5

4.3. Path analysis

Simple Pierson correlations in such circumstances may well not clear relevance between traits and so the breeders use direct and indirect users of strategy selection and use for decision making. Path analysis helps to explain direct and indirect effects and so widely in breeding programs used by researchers in different plant species (Aguado *et al*., 2010). For better evaluation and more accurate interpretation of the results obtained from simple correlations and stepwise regression, the traits entered in the final regression model were path analyzed. The results of path analysis showed that biological yield (0.785) had most direct positive effect on seed yield. It seems increasing amount of biological yield due to the provision of photosynthetic materials (source products) and cooperate of seeds (sink) to receive and accumulate total dry matter through the number of pods per plant (0.130), number of seeds per pod (0.025), weight of 1000-seeds (-0.030) and pod length (0.05) have indirect positive effects on seed yield (Table 5).

Table 5. Path analysis of yield traits between remaining in the stepwise regression model

Trait	Direct effect	Indirect effect					Total effect
		Biologic yield	Number of pod per plant	Number of seed per pod	1000-seed weight	Pod length	
Biologic yield	0.785	----	0.130	0.025	-0.030	0.05	0.960
Number of pod per plant	0.656	0.17	----	0.097	-0.037	0.045	0.931
Number of seed per pod	0.112	0.645	0.150	----	-0.037	0.035	0.905
1000-seed weight	0.430	.257	0.049	0.051	----	0.047	0.834
Pod length	0.156	0.476	0.134	0.074	-0.058	----	0.782

Error= 0.54 (Residual effect)

Variable of number of pods per plant with direct positive effect (0.656) on seed yield with indirect positive effects of biological yield (0.17), number of seeds per pod (0.097), 1000-seed weight (-0.037) and pod length (0.045) caused a positive correlation between this variable and seed yield (Table 5). It seems the sink is a large storage capacity, by more number of seed led to achieve higher crop production. Changing the number of seeds in the pods potentially increases seed yield because source done photosynthesizes and increases the capacity of sink or material storage in the crop. Characteristics that have an update priority can have direct effects on crop production and have an indirect effect on yield through other variables that are found in the later stages of plant growth and development (Artin, 2015). Variable of the number of seeds per pod with a positive direct effect (0.112) on seed yield with indirect effects of biological yield (0.645), number of pods per plant (0.150), 1000-seed weight (0.037) 0) and pod length (0.035) caused a positive correlation between this trait and seed yield (Table 5). Ismaili *et al*. (2017) by evaluate relation between canola genotypes reported the most important traits in order to select index for grain yield and oil yield improvement under stress condition were pod length and grain yield, respectively. Therefore, selection based on these traits would be more effective to improving seed yield of canola in well-watered and water-deficit stress conditions. So, the method of path coefficients proved useful in analyzing correlation coefficients in this system of interrelated variables. 1000-seed weight variable with a positive direct effect (0.430) on seed yield with indirect positive effects of biological yield (0.257), number of pods per plant (0.049), number of seeds per pod (0.051) and pod length (0.047) caused a positive correlation between this variable and seed yield (Table 5). Pod length variable with a positive direct effect (0.156) on seed yield with indirect positive effects of biological yield (0.476), number of pods per plant (0.134), number of seeds per pod (0.074) 1000-seed weight (-0.058) caused a positive correlation between this variable and seed yield (Table 5).

5. CONCLUSION

All the studied traits were put into regression model and finally five traits of biologic yield, number of pod per plant, number of seed per pod, 1000-seed weight and pod length remained in the regression model. The mentioned model generally determines 93.5 percent of variation related to the seed yield. So breeders can use selected traits for achieve optimum genotypes instead of direct selection for seed yield.

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FOOTNOTES

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