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Evaluation Effect of Different Amount and Time of Apply Gibberellin Acid on Correlation between Trait, Regression Relationships and Grain Filling Rate of Broad Bean

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ABSTRACT

BACKGROUND: *Vicia faba* L. is a major crop legume that is used as food owing to the high nutrient components in seeds. Development of high yielding and stable cultivars of various legume crops across different environments is very important for their adoption by farmers.

OBJECTIVES: The current study was conducted to evaluate the effect of different amount and time of apply Gibberellin acid on correlation between characteristics and regression relationship between effective traits on seed yield of Broad bean.

METHODS: This research was done via factorial experiment based on randomized complete blocks design with three replications along 2010 year. The treatments included different concentration of Gibberellin (nonuse of Gibberellin or control, 5 ppm, 50 ppm and 250 ppm) and time of application Gibberellin (Vegetative growth before flowering, flowering until pod emergence, Pod emergence until grain filing).

RESULT: According result of analysis of variance effect of different Gibberline acid concentration at different growth stage and interaction effect of treatment on all measured traits was significant. Correlation between traits showed the significant correlation between total dry weight (r=0.97**), harvest index (r=0.94**), seed weight (r=0.90**), number of seed per pod (r= 0.85**), number of pod per plant (r= 0.81**), Pod length (r= 0.61**), leaf area index (r= 0.59**) and seed yield was observed. Grain filling rate changes in all three concentrations of gibberellin acid had an increasing trend. Gibberellin acid consumption in the period of vegetative growth compared to apply hormone at other growth stage had the highest grain filling rate.

CONCLUSION: The highest grain filling rate achieved by use of 50 ppm Gibberellin acid at vegetative growth before flowering.

KEYWORDS: *Bio-Regulator, Nutrition, Pulse, Seed yield, Vicia faba.*

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1. BACKGROUND

Broad bean is a cool-season grain legume crop originated in the Middle East in the pre-historic times and traditionally used as a main source of protein for human and animal nutrition (Multari *et al*., 2015). Broad bean is considered as one of the most important pulse crops in the world. In recent years, cultivation of broad bean has received large attention in USA, Canada and Europe (Etemadi *et al*., 2018). Bean is one of the most important grain legumes with the cultivated area of 105113 hectares and average tonnage of 21000 $hg.ha^{-1}$ Iran In 2018 (FAO, 2020). The major cultivation areas include Gorgan, Khuzestan, Lorestan, Hormozgan and Gilan (Sharifi *et al*., 2016). Like other crops, variety or cultivar selection requires achieving a balance between adaptability to a specific environment, disease tolerance, purpose of cultivation and marketability (Zandvakili *et al*., 2019). Multivariate analyses are useful for characterization, evaluation and classification of plant genetic resources when a number of accessions are to be assessed for several characters of agronomic, morphological and physiological importance. Different types of multivariate analysis such as regression analysis, path analysis, principal component analysis (PCA) can be used to identify groups of genotypes that have beneficial traits for breeding and instructing the patterns of variation in genotype accession, to recognize relationships among accessions and possible gaps. Correlation coefficients describe the mutual relationships between different pairs of characters without providing the nature of cause and effect relationship of each character (Sharifi *et al*., 2020). Yield improvement is a major breeding objective of most crop improvement programs (Ghobary and Abd-Allah, 2010). Yield in broad bean, similar to the other crops, is a complex trait and constitute by many of morphological and physiological traits. Seed yield is affected by genotype and environmental factors because it is a quantitative trait. Using as selection criteria of characters, direct relationship with seed yield increase the success of selection in plant breeding (Karasu and Oz, 2010). Therefore, progress of breeding in such traits are primarily conditioned by the magnitude and nature of variation and interrelationships among them (Raffi and Nath, 2004). The breeding objectives for this crop are grain yield and grain yield stability and lodging resistance, and furthermore resistances against drought (winter frost in case of winter bean breeding), and against fungi and further pathogens and pests, with additional objective of underground root dynamic and grain quality (Singh *et al*., 2012). Correlation analysis describes the mutual relationship between different pairs of characters without providing the nature of cause and effect relationship of each character. Significant positive correlations were detected between faba bean seed yield and each of number of pods per plant, number of seeds per plant, seed weight per plant and biological yield (Alghamd, 2007). Plant growth regulators are known to influence growth and development at very low concentrations but inhibit plant growth and development at high concentrations (Jules *et al*. 1981). Several researches have shown the stimulatory effects of growth regulators on the vegetative growth and yield of plants. Gibberellic acid has been used to stimulate stem and petiole extension in rhubarb, celery and water cress (Thomas, 1976). Treatment 3 of radish and onion seeds with auxin or a mixture of gibberellic acid (GA) and kinetin have been found to increase the germination of the seeds (Thomas, 1976). Monthly foliar spraying of geranium (*Pelergonium graveolens*) resulted in increased plant height and herb production (Mohammed *et al.*, 1983). Gibberellins play a role in balancing the growth of internodes and the growth and development of the leaves. It has been found that plants grow in the long-day or Chilling requirement if they remain in the short day or in warm conditions, where it remain growing vegetative and do not flowering. The treatment of these plants with gibberellic acid will be compensated by the requirements of the light or cold period and thus elongate the stems, flowering and the plants where the elongation of the flower stem and flowered They contain more gibberellin compounds than plants that have not elongation of the flower stem and non-flowering stems (Saleh, 1990). Gibberellins (GAs) are growth hormones strongly involved in a wide variety of physiological activities. Currently, gibberellins are commercially used to enhance phenotypic characteristics, earliness, and productivity of many vegetable and ornamental crops (Miceli *et al*., 2019). Fadhil and Almasoody (2019) by evaluated effect of spraying with Gibberellic Acid on broad bean cultivars reported the highest average of plant height, number of branches in the plant, the number of pods in the plant, while the Italian genotype gave the highest average of the pod length and the number of seeds per pod, weight of 100 gram seed, the plant yield and total plant yield was related to apply 300 mg. L^{-1} gibberellic acid.

2. OBJECTIVES

The current study was conducted to evaluate the effect of different amount and time of apply Gibberellin acid on correlation between characteristics and regression relationship between effective traits on seed yield and grain filling rate of Broad bean.

3. MATERIALS AND METHODS

3.1. Field and Treatments Information

This research was carried out via factorial experiment based on randomized complete blocks design with three replications along 2010 year. 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 treatments included different concentration of Gibberellin (nonuse of Gibberellin or control, 5 ppm, 50 ppm and 250 ppm) and time of application Gibberellin (Vegetative growth before flowering, flowering until pod emergence, Pod emergence until grain filing). This experiment had 36 plots. Each plot consisted of 7 lines with a distance of 60 cm and 5 meters length. The distance between the shrubs on every row was 15 cm.

3.2. Farm Management

Base fertilizers (75 kg.ha^{-1} Nitrogen from urea, 100 kg.ha^{-1} phosphorus from ammonium phosphate and 80 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 50 kg.ha^{-1} nitrogen added at stem elongation stage. Physical and chemical properties of studied soil were mentioned in table 1. To combat the weeds during the growth, weeding and thinning was done manually.

Table 1. Physical and chemical properties of studied field

Soil depth (cm)	**SP (%)**	**EC (ds.m^{-1})**	**pH**	**TNV (%)**
0-30	50	11.48	7.95	36
30-60	51	12.1	7.9	39
Soil depth (cm)	**N (%)**	**P (ppm)**	**K (ppm)**	**Sand (%)**
0-30	0.07	4.6	202	14
30-60	0.052	4.0	171	12
Soil depth (cm)	**OM (%)**	**OC (%)**	**Silt (%)**	**Clay (%)**
0-30	1.24	0.72	53	33
30-60	0.95	0.55	57	31

3.3. Measured Traits

Characteristics such as plant height, node number, node length, stem number and pod length was measured. In final harvest area, one- square meter of each plot, seed yield were calculated. In addition, seed samples were dried and weighed. In order to determine the leaf and stem dry weight two planting lines from each plot harvested and after the removal of marginal effect were carried to the laboratory and were placed in the oven at 75°C for 48 hours and after ensuring that the samples were completely dry, they were weighed and finally the dry matter was measured. 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.

Harvest index (HI) was calculated according to formula of Gardener *et al.* (1985) as follows: **Equ.2.** HI= (Seed yield/Biologic yield) ×100.

To determine the leaf area of the linear relationship S= K. L.W was used in which S, L and W were the leaf area, L and W respectively, the maximum length and width of each leaf and K= 0.75 correction coefficient. The leaf area index was calculated from leaf area ratio to ground level.

3.4. Statistical Analysis

All data analyzed via MSTAT-C software and determine correlation between traits by Minitab software (Ver.15). Regression relationship was drawn with Excel software (Ver.2010).

4. RESULT AND DISCUSSION

4.1. *Result of Analysis of variance*

4.1.1. *Number of pod per plant*

Result of analysis of variance revealed effects of different amount, time of apply Gibberellin acid and interaction effect of treatments on number of pod per plant was significant at 1% probability level (Table 2).

Table 2. Result analysis of variance of measured traits

S.O.V	df	No. pod per plant	No. seed per pod	Seed weight	Seed yield	Total dry weight	Harvest index
Replication	2	ns	ns	ns	ns	ns	ns
Gibberline concentration (GC)	3	**	**	**	*	*	**
Growth stage (GS)	2	**	**	**	*	*	**
GC × GS	6	**	**	**	*	*	**
CV (%)	-	10.24	9.65	7.6	11.17	11.33	7.1

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

Continue table 2.

S.O.V	df	Leaf area index	Stem dry weight	Leaf dry weight	Protein content	Nitrogen content
Replication	2	ns	ns	ns	ns	ns
Gibberline concentration (GC)	3	*	**	**	*	*
Time of application Gibberline (GS)	2	*	**	**	*	*
GC × GS	6	*	**	**	*	*
CV (%)	-	10.24	7.05	7.09	9.67	8.1

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

Continue table 2.

S.O.V	df	Plant height	Node number	Node lenght	Stem number	Pod length
Replication	2	ns	ns	ns	ns	ns
Gibberline concentration (GC)	3	**	**	*	*	*
Growth stage (GS)	2	**	**	*	*	*
GC × GS	6	**	**	*	*	*
CV (%)	-	9.74	7.10	9.63	10.44	10.57

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

4.1.2. *Number of seed per pod*

According result of analysis of variance effect of different amount, time of applies Gibberellin acid and interaction effect of treatments on number of seed per pod was significant at 1% probability level (Table 2).

4.1.3. *Seed weight*

Result of analysis of variance revealed effects of different amount, time of apply Gibberellin acid and interaction effect of treatments on seed weight was significant at 1% probability level (Table 2).

4.1.4. *Seed yield*

Result of analysis of variance indicated effects of different amount, time of apply Gibberellin acid and interaction effect of treatments on seed yield was significant at 5% probability level (Table 2).

4.1.5. *Total dry weight*

According result of analysis of variance effect of different amount, time of applies Gibberellin acid and interaction effect of treatments on total dry weight was significant at 5% probability level (Table 2).

4.1.6. *Harvest index*

Result of analysis of variance indicated effects of different amount, time of apply Gibberellin acid and interaction effect of treatments on harvest index was significant at 1% probability level (Table 2). It seems the harvest index trait generally had lower affected environment situation.

4.1.7. *Leaf area index*

According result of analysis of variance effect of different amount, time of applies Gibberellin acid and interaction effect of treatments on leaf area index was significant at 5% probability level (Table 2).

4.1.8. *Stem dry weight*

According result of analysis of variance effect of different amount, time of applies Gibberellin acid and interaction effect of treatments on stem dry weight was significant at 1% probability level (Table 2).

4.1.9. *Leaf dry weight*

Result of analysis of variance showed effects of different amount, time of applies Gibberellin acid and interaction effect of treatments on leaf dry weight was significant at 1% probability level (Table 2).

4.1.10. *Protein content*

According result of analysis of variance effect of different amount, time of applies Gibberellin acid and interaction effect of treatments on protein content was significant at 5% probability level (Table 2).

4.1.11. *Nitrogen content*

Result of analysis of variance showed effects of different amount, time of applies Gibberellin acid and interaction effect of treatments on nitrogen content was significant at 5% probability level (Table 2).

4.1.12. *Plant height*

According result of analysis of variance effect of different amount, time of applies Gibberellin acid and interaction effect of treatments on plant height was significant at 1% probability level (Table 2).

4.1.13. Node number

Result of analysis of variance revealed effects of different amount, time of apply Gibberellin acid and interaction effect of treatments on node number was significant at 1% probability level (Table 2).

4.1. 14. Node length

According result of analysis of variance effect of different amount, time of applies Gibberellin acid and interaction effect of treatments on node length was significant at 5% probability level (Table 2).

4.1.15. Stem number

Result of analysis of variance revealed effects of different amount, time of apply Gibberellin acid and interaction effect of treatments on stem number was significant at 5% probability level (Table 2).

4.1.16. Pod length

According result of analysis of variance effect of different amount, time of applies Gibberellin acid and interaction effect of treatments on pod length was significant at 5% probability level (Table 2).

4.2. *Correlation between traits*

Knowing the relationship among these processes and investigating other quantitative traits make breeding programs and their success more optimistic and secure (Menapour *et al*., 2006). Seed yield is a quantitative trait, which expression is the result of genotype, environmental effect and genotype-environment interaction (Gunasekera *et al*., 2006). Simple correlation coefficients between studied traits were estimated according to Pearson coefficient. Result showed the significant correlation between total dry weight ($r=0.97^{**}$), harvest index ($r=0.94^{**}$), seed weight ($r=0.90^{**}$), number of seed per pod ($r= 0.85^{**}$), number of pod per plant ($r= 0.81^{**}$), Pod length ($r= 0.61^{**}$), leaf area index ($r= 0.59^{**}$) and seed yield was observed (Table 3). Tadesse *et al*. (2011) indicated number of pods per plants, number of seeds per pod, thousand seed weight and plant height had significant association with seed yield per plot. The seed yield per plant exhibited positive and significant correlation with clusters per plant, pod length, plant height, branches per plant, pods per plant and hundred seed weight (Badolay *et al*., 2009). Ulukan *et al*. (2003) also found positive and significant relationships between biological yield and plant height and grain number per pod. Keneni and Jarso (2002) indicated positive and significant correlation between Seed yield and number of pods per plant. Sharifi *et al*. (2020) by compare faba bean (*Vicia faba* L.) genotypes by multivariate analyses reported the analysis of variance showed significant differences between genotypes for all of the studied traits. Correlation analysis indicated there were positive correlation coefficients between seed yield and number of days to germination, number of days to flowering, plant height, number of pods per plant, number of nodes per stem, hundred seed weight, pod length, biological yield and harvest index. Regression analysis indicated seed yield as dependent variable, while plant height, number of pods per plant, number of stems per plant, number of nodes per stem, number of seeds per pod, hundred seed weight and pod length were considered as casual variables. Attention should be paid to traits such as plant height, number of pods per plant, number of nodes per stem and pod length for augmentation of seed yield and these traits could be used as selection criteria in faba bean breeding programs.

4.3. *Regression relationship*

In Stepwise regression analysis, grain yield was considered as a dependent variable, while other traits were considered as independent variables (Shiapchan, 2012). Quantification of plant characteristics associated with yield increase is important in crop breeding programs. Regression models can be used for this purpose (Jafarnodeh *et al.*, 2017).

Equ.3. Y= -1895 + 15.9 NPP + 22.5 NSPP + 11.2 SW-13.6 SY + 28.5 TDW + 1.79 HI- 2.42 LAI + 5.11SDW+ 7.02 LDW + 118 PC + 101 NC + 9.05 PH+ 144 NN+ 133 NL+87 SN+45.11 PL.

NPP: Number of pod per plant, NSPP: Number of seed per pod, SW: Seed weight, SY: Seed yield, TDW: Total dry weight, HI: Harvest index, LAI: Leaf area index, SDW: Stem dry weight, LAI: Leaf dry weight, PC: Protein content, NC: Nitrogen content, PH: Plant height, NN: Node number, NL: Node length, SN: Stem number, PL: Pod length.

Fig. 1 and degree of correlation ($r = 0.98^{**}$) show a strong and positive correlation between this factor and seed yield. Because the increase in seed yield is followed by an increase in total dry matter, stem length and diameter, number of nodes and pods per unit area for the bean plant, so any increase in this factor will have a positive effect on seed yield.

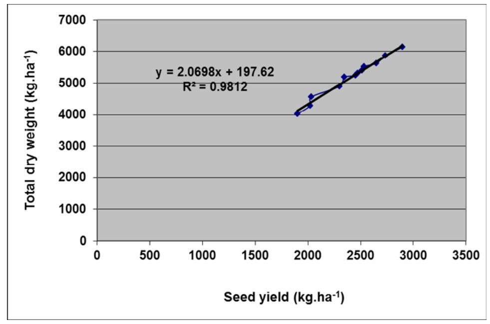

Fig. 1. Regression relationship between seed yield and total dry weight.

Jafarnodeh *et al* (2017) reported regression model revealed that faba been seed yield can be increased from 1665 $kg.ha^{-1}$ to 2880 $kg.ha^{-1}$ (1215 $kg.ha^{-1}$ increase). They are find this increase can be achieved by manipulation of four plant traits, i.e., plant height, pod number per plant, seed number per pod, and days to flowering. There was no significant negative correlation between the traits. Optimizing of these traits, within the observed range in the field experiment, can increase faba bean seed yield by 1215 $kg.ha^{-1}$. The contribution of each trait in this increase was estimated: plant height by 503 $kg.ha^{-1}$, pod number per plant by 344 $kg.ha^{-1}$, seed number per pod by 327 $kg.ha^{-1}$ and days to flowering by 41 $kg.ha^{-1}$.

4.4. *Grain Filling Rate*

Grain filling rate changes in all three concentrations of gibberellin acid had an increasing trend. According to Fig. 2, comparison of different concentrations of gibberellin showed that the concentration of 50 ppm and then 250 ppm had the highest increase in grain filling rate and in contrast lack of hormone consumption and concentration 5 ppm showed lowest rate of grain filling rate.

Table 3. Correlation between studied traits

Trait	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	-														
2	-0.71**	-													
3	0.69**	-0.65*	-												
4	0.81**	0.85**	0.90**	-											
5	0.74**	0.75**	0.80**	0.97**	-										
6	0.51*	0.52*	0.69**	0.94**	0.77**	-									
7	0.48^{ns}	0.49^{ns}	0.44^{ns}	0.59*	0.82**	0.47^{ns}	-								
8	0.40^{ns}	0.42^{ns}	0.41^{ns}	0.42^{ns}	0.88**	0.59*	0.58*	-							
9	0.42^{ns}	0.44^{ns}	0.45^{ns}	0.38^{ns}	0.85**	0.52*	0.88**	0.81**	-						
10	0.34^{ns}	0.39^{ns}	0.31^{ns}	0.32^{ns}	0.22^{ns}	0.21^{ns}	0.23^{ns}	0.30^{ns}	0.34^{ns}	-					
11	0.36^{ns}	0.30^{ns}	0.33^{ns}	0.28^{ns}	0.50*	0.32^{ns}	0.27^{ns}	0.35^{ns}	0.38^{ns}	0.91**	-				
12	0.50*	0.51*	0.47^{ns}	0.46^{ns}	0.48^{ns}	0.55*	0.25^{ns}	0.53*	0.51*	0.27^{ns}	0.39^{ns}	-			
13	0.52*	0.55*	0.51*	0.45^{ns}	0.51*	0.23^{ns}	0.29^{ns}	0.49^{ns}	0.47^{ns}	0.35^{ns}	0.49^{ns}	0.77**	-		
14	0.45^{ns}	0.48^{ns}	0.55*	0.33^{ns}	0.49^{ns}	0.28^{ns}	0.31^{ns}	0.47^{ns}	0.49^{ns}	0.46^{ns}	0.40^{ns}	0.48^{ns}	0.85**	-	
15	0.53*	0.57*	0.39^{ns}	0.31^{ns}	0.57*	0.45^{ns}	0.28^{ns}	0.89**	0.78**	0.64*	0.52*	-0.56*	0.79**	0.65*	-
16	0.59*	0.55*	0.58*	0.61*	0.52*	0.41^{ns}	0.46^{ns}	0.66*	0.40^{ns}	0.51^{ns}	0.50*	0.33^{ns}	0.39^{ns}	0.31^{ns}	0.29^{ns}

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

1: Number of pod per plant, **2**: Number of seed per pod, **3**: Seed weight, **4**: Seed yield, **5**: Total dry weight, **6**: Harvest index, **7**: Leaf area index, **8**: Stem dry weight, **9**: Leaf dry weight, **10**: Protein content, **11**: Nitrogen content, **12**: Plant height, **13**: Node number, **14**: Node lenght, **15**: Stem number, **16**: Pod length.

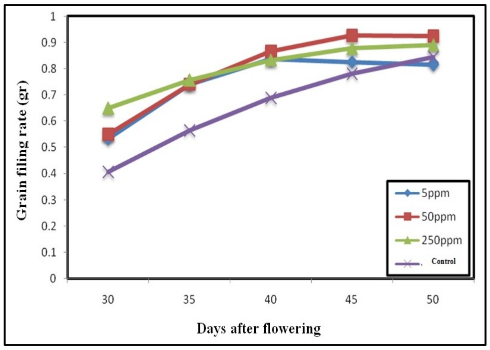

Fig. 2. Effect of different concentration of Gibberellin acid on grain filling rate

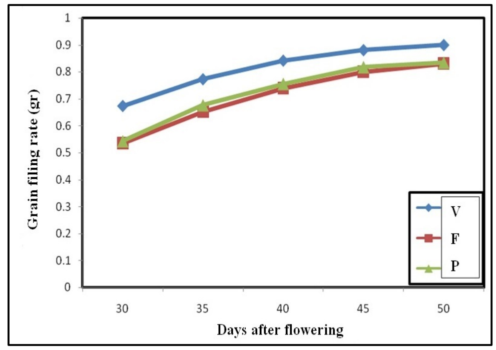

Fig. 3. Effect of different time of apply Gibberellin acid on grain filling rate
V: Vigitative growth stage, **F**: Flowering stage, **P**: poding growth stage

Factors that control sink power and grain filling rate (GFR) can control the distribution of photosynthetic materials. Hormones can have a significant effect on photosynthetic material transfer and grain filling through their effect on enzymatic activity and flexibility of target cells, so mentioned effect is more observed in high concentrations of gibberellin acid (Daglans, 2014). Fig. 3 showed that trend of changes in GFR at studied growth stages was an upward trend. The reason for decrease in process in harvest and ripening stage was loss of moisture in seeds. Gibberellin acid consumption in period of vegetative growth compared to apply hormone at other growth stage had highest GFR. It seems that when the plant receives the gibberellin acid during the vegetative growth period, due to the positive effect that gibberellin on division and growth and expansion of various organs of the plant, the production of photosynthetic materials also increases, which leads to increased grain filling rate (Fitals, 2016). The flowering period and pod emergence was not greatly affected by gibberellin because the plant had reached its maximum growth.

5. CONCLUSION

Effect of different Gibberline acid concentration at different growth stage and interaction effect of treatment on all measured traits was significant. The highest grain filling rate achieved by use of 50 ppm Gibberellin acid at vegetative growth before flowering.

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FOOTNOTES

AUTHORS' CONTRIBUTION: All authors are equally involved.

CONFLICT OF INTEREST: Authors declared no conflict of interest.

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