



Effect of micronutrient and irrigation deficit on yield and yield components of isabgol (*Plantago ovata* Forsk) using multivariate analysis

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Received 30 October 2010, accepted 5 January 2011.

Abstract

In order to affect micronutrient and irrigation deficit on yield and component yield of isabgol, experiment was carried out based on split-plot design with based design of random complete block. Main plots were irrigation deficit and secondary plots were application of micronutrients. Means of traits were used for multivariate analysis, such as multiple regression, stepwise regression, principle component analysis and path analysis for mucilage yield. Variance analysis of traits showed grain yield, biological yield and 1000-kernel weight were influenced by both limited irrigation and micronutrients. Mucilage yield and number of leaves per plant were influenced by micronutrients, and number of spikes per plant was influenced by limited irrigation. As mucilage yield, treatments that received Zn to concentration of 3 ppt were better than treatments that received Mn, Fe and complete mixture of Zn, Fe and Mn. Mucilage yield had significant positive correlation with grain yield and biological yield. Stepwise regression of grain yield showed that number of spikes per plant was the first variable entered to model and contributed 37.6% of grain yield variation. Then 1000-grain weight was second variable entered to model and overall number of spikes per plant explained 54.4% of variation and in step 3 harvest index entered to model explained overall 75.4% of variation. In the correlation analysis number of spikes per plant also had significant positive correlation with grain yield. The multiple correlation coefficient for mucilage yield was 0.999 and in stepwise regression, harvest index, number of leaves per plant and number of grain per plant entered to model, respectively, explained overall 0.999 of mucilage yield variation. Results of path analysis showed grain yield had highest effect on mucilage yield. Number of spikes per plant via influence of grain yield and biological yield had highest indirect effect to mucilage yield. Therefore, in order to increase mucilage yield it is favorable to select plants according to grain yield and of number of spikes per plant. PCA analysis for mean of factors interaction (A and B) showed that 3 PCs with eigenvalues higher than 1 accounted for 88% of the total variation. PC₁, PC₂ and PC₃ explained 54.6%, 24.8% and 0.08% of the total variation, respectively.

Key words: Isabgol, micronutrient, irrigation deficit, multivariate analysis.

Introduction

Medicinal plants are the most important source of life saving drugs for the majority of world's population. Plant secondary metabolites are economically important as drugs, fragrances, pigments, food additives and pesticides. Isabgol (*Plantago ovata* Forsk) belongs to Plantaginaceae family and is an important medicinal plant. Its English name is blond psyllium, spogel seeds and its Indian names are snigdhajirah, snigdhajirakah (Sanskrit) isabgol, isabgul (Hindi), iskol, isphogol (Tamil). India is the largest producer and exporter of this crop in the world. The seeds of isabgol contain mucilage components that are used in industries of medicine, foods and oil. The mucilage is obtained from the seed coat by mechanical milling/grinding of the outer layer of the seeds. It is a white fibrous hydrophilic material which forms a clear colorless mucilaginous gel by absorbing water. The gel nature and composition of the polysaccharides extracted from the seeds of the *P. ovata* has been reported in literature^{9, 15}. *Plantago ovata*, popularly known as isabgol, has great commercial importance due to thin rosy white membranous seed husk¹⁸. The seed husk is used to cure inflammation of the mucous membrane of gastro-intestinal and genitourinary tract, chronic constipation, dysentery, duodenal ulcers, gonorrhoea and piles^{5, 21}. Numerous studies of nondiabetic individuals indicate that *Plantago ovata*

significantly lowers both total and LDL-cholesterol concentrations^{2, 3}. Anderson *et al.*¹ found that addition of *Plantago ovata* to a traditional diet for persons with diabetes is safe, well tolerated, and improves glycemic and lipid control in men with type 2 diabetes and hypercholesterolemia. Trace elements or micronutrients and secondary nutrients are absolutely essential for plant growth. The need for these nutrients varies widely with crop, soil conditions and farm management, and with the levels of nitrogen, phosphate and potassium. Constant attention should be given to micronutrient levels and needs. A crop can suffer from nutrient deficiency without showing a specific symptom. If the symptom is evident, the crop is already suffering a deficiency and it will result on yield reduction. Micronutrients that we have used were Fe, Mn, Zn. Fe function within the plant is promoting formation of chlorophyll, Mn is a part of important enzymes involved in respiration and protein synthesis and Zn is important as a catalyst for plant growth regulators in plants, and the use of other nutrients affects maturity¹⁹. Sabagh-Nekoum *et al.*¹⁴ investigated the effect of plant density on yield and yield components of *Plantago ovata* Forsk and found significant differences among the accessions for their seed yield, yield component and effective medicine ingredients. Although

genetics and seasonally induced changes are the primary determinants of the composition of plant secondary metabolites, the mucilage content may also be affected by environmental factors and the fertilization regime. Some authors propose that variation in concentrations of secondary metabolites may be due to differences in plant growth and accumulation of biomass²⁰. Application of organic matter positively affects the growth and development of plant roots and shoots⁷. Singh *et al.*¹⁶ reported that the total dry matter, seed and mucilage yields of isabgol can be increased with application of chemical and organic fertilizers. In this study we investigated effect of micronutrients in isabgol under irrigation limited. Our objective was investigation of effect of micronutrients (Fe, Zn, Mn) in the mucilage yield, biological and economical yield.

Materials and Methods

Isabgol (*Plantago ovata* Forsk) plants were grown under different fertilization regimes. The experiment was carried out in Zabol University research field located in Chahnimeh (30°55' N, and 483 m above the sea level). Soil texture was loamy sandy. Sowing occurred in the spring 2008, in a greenhouse. Plantlets were transferred to field and experiment was carried out based on split-plot design to based design random complete block with four replications. Main plots were: a₁ common irrigation, a₂ delay of irrigation before flowering stage and a₃ delay of irrigation after flowering stage. Secondary plots were application of micronutrients (b₁ control treatment, b₂ Fe to concentration of 4 ppt, b₃ Zn to concentration of 3 ppt, b₄ Mn to concentration of 4 ppt and b₅ complete mixture of b₂, b₃ and b₄). Application of micronutrients was conducted in two steps: before flowering stage and after flowering stage. From each secondary plot 1 m² was harvested to determine biological and economical yield after complete maturity of plants. Mucilage percentage and swelling index according to Ebrahimzadeh *et al.*⁶ was measured.

Means of traits were used for multivariate analysis. Analysis of variance, simple correlation of traits, multiple regression, stepwise regression and PCA were conducted using SAS software version 9.1¹⁶. Path analysis was carried out for mucilage yield. Chart of biplot was conducted using NTSYS - PC version 2.2¹².

Results and Discussion

Variance analysis of traits showed that grain yield, biological yield and 1000 kernel weight were influenced by both limited irrigation and micronutrient. Mucilage yield and number of leaves per plant were influenced only by micronutrient and number of spikes per plant was influenced only by limited irrigation. The interaction of factors did not show significant differences in any of the traits (Table 1).

Comparison of traits mean in irrigation limited condition (factor A) showed that grain yield, biological yield, 1000 grain weight, number of spikes per plant and number of grains per plant had significant difference in common irrigation condition (a₁) to delay of irrigation before flowering stage (a₂) and delay of irrigation after flowering stage (a₃) (Table 2).

Mean comparison for micronutrient applications (factor B) indicated treatments were divided in two groups in grain yield, biological yield, mucilage yield, number of grains per plant and number of leaves per plant and in three groups in 1000-grain weight (Table 3).

Table 1. Variance analysis of traits in the isabgol medical plant.

Characters	MS of S.O.V			
	a	r*a	b	a*b
X ₁	80561.43**	11824.711	20558.686**	390.159
X ₂	546423.861*	67808.12	129870.22*	1855.58
X ₃	1878.565	419.38	795.8*	13.603
X ₄	6.308	2.77	5.53	2.91
X ₅	0.0846**	0.0073	0.0701*	0.00404
X ₆	0.624	2.166	0.828	0.298
X ₇	12.12	8.108**	0.141	0.748
X ₈	0.176	0.574**	0.103	0.0444
X ₉	25.84**	1.186	4.94	2.43
X ₁₀	107.228*	14.58	251.95**	5.094
X ₁₁	0.766	1.375*	0.507	0.777
X ₁₂	0.024	0.127	0.137	0.077
X ₁₃	327.603	366.087**	162.32*	96.35
X ₁₄	27.91	8.02*	2.47	2.5

*and ** significant at 5% and 1% levels, respectively.

X₁ = grain yield, X₂ = biological yield, X₃ = mucilage yield X₄ = harvest index, X₅ = 1000-grain weight, X₆ = mucilage, X₇ = plant height, X₈ = spike height, X₉ = number of spikes per plant, X₁₀ = number of grains per plant, X₁₁ = swell index, X₁₂ = number of forks in each plant, X₁₃ = number of leaves in each plant, X₁₄ = number of days from flowering to maturity.

Table 2. Comparison of traits mean for a (irrigation limited).

a/traits	X ₁	X ₂	X ₅	X ₉	X ₁₀
common irrigation	610.74 A	1813.61 A	1.81 A	26.63 A	81.69 A
delay of irrigation before flowering stage	521.64 B	1604.94 B	1.74 B	24.81 B	79.72 AB
delay of irrigation after flowering stage	487.9 B	1487.23 B	1.68 B	24.52 B	77.08 B

As mucilage yield, treatments that received Zn in concentration of 3 ppt were better than treatments that received Mn in concentration of 4 ppt, Fe in concentration of 4 ppt and complete mixture of Zn, Fe and Mn. Control treatment had the lowest mucilage yield. The results showed application of Zn in concentration of 3 ppt and then Mn in concentration of 4 ppt and complete mixture of Zn, Fe and Mn increased mucilage yield, respectively (Table 4). Therefore we can say, the use of micronutrients increased mucilage yield and secondary metabolites. These results are in agreement with Pouryousef *et al.*¹¹, who investigated the effect of different soil fertilizing systems on seed and mucilage yield and seed P content of isabgol. Their results were that Barvar phosphate biofertilizer (BPB) inoculation significantly increased seed yield, mucilage yield and seed P content and also animal manure and combined use of manure and chemical fertilizer resulted in a greater seed yield, mucilage yield, mucilage percentage, swelling factor and P content than application of chemical fertilizer. In studies by Sabagh-Nekonom and Razmjoo¹⁴ in order to influence plant density on yield, yield components and pharmaceutical properties of isabgol, plant density did not shown significant difference between yield components and pharmaceutical properties of isabgol. Hendawy⁸ used organic and mineral fertilization for *Plantago orenaria* plant

Table 3. Comparison of traits for b (micronutrient application).

b/ traits	X ₁	X ₂	X ₃	X ₅	X ₁₀	X ₁₃
Control treatment	472.6 B	1455.3 B	76.215 B	1.633 C	73.94 C	58.8 AB
Fe to concentration of 4 ppt	549.3 A	1682.2 AB	92.88 AB	1.733 B	84.07 A	62.94 A
Zn to concentration of 3 ppt	577.18 A	1716 A	96.67 A	1.8 AB	84.41 A	53.71 B
Mn to concentration of 4 ppt	568.2 A	1681.7 AB	94.312 AB	1.83 A	77.2 B	54.56 B
Complete mixture of b ₂ , b ₃ and b ₄	533.18 A	1641 A	87.85 AB	1.74 B	77.85 B	58.08 AB

Table 4. Means of factors intraction (A and B) in isabgol under micronutrient application and irrigation deficit.

t/traits	X1	X2	X3	X4	X5	X6	X7	X8	X9	X10	X11	X12	X13	X14
a1b1	524.70	1634.73	84.29	32.08	1.75	16.03	22.82	3.43	25.83	75.58	9.93	3.40	63.50	56.25
a1b2	499.60	1601.70	103.97	414.97	9.73	13.33	20.52	8.70	20.94	71.98	31.25	5.18	52.35	60.17
a1b3	650.45	1895.85	107.98	34.21	1.88	16.55	23.30	3.23	26.48	85.45	10.70	3.15	50.71	54.50
a1b4	640.70	1879.50	105.53	34.16	1.90	16.42	22.79	3.37	26.85	79.15	9.45	3.10	61.35	55.50
a1b5	608.25	1797.23	98.64	33.85	1.78	16.17	22.99	3.24	26.80	80.13	10.38	2.85	55.25	55.25
a2b1	463.20	1451.23	74.44	31.90	1.63	16.17	21.82	3.14	24.00	71.55	10.13	3.10	53.10	58.00
a2b2	524.45	1644.38	91.03	31.99	1.73	17.39	21.11	3.07	25.75	80.05	10.33	3.10	55.00	58.00
a2b3	562.18	1664.68	94.47	33.86	1.80	16.81	20.71	3.02	23.65	83.30	10.83	3.15	55.75	57.00
a2b4	549.60	1638.98	91.02	33.64	1.83	16.55	22.10	3.46	24.30	74.85	10.05	2.90	47.60	56.25
a2b5	508.78	1625.45	83.62	31.25	1.75	16.40	21.41	3.11	25.00	75.65	10.25	3.15	53.35	56.25
a3b1	429.90	1280.05	69.91	33.64	1.53	16.23	22.00	3.24	22.85	74.70	10.63	3.20	59.80	54.75
a3b2	493.85	1541.50	82.37	31.93	1.70	16.40	22.53	3.38	24.70	84.00	10.50	3.10	64.74	54.50
a3b3	518.90	1587.53	87.59	32.68	1.73	16.93	22.64	3.21	26.15	84.50	10.50	3.30	54.85	53.75
a3b4	514.30	1526.65	86.40	33.82	1.78	16.82	22.48	3.32	25.25	77.60	11.15	2.90	54.75	55.25
a3b5	482.53	1500.40	81.30	32.14	1.70	16.72	22.37	3.21	25.10	77.80	10.33	3.20	65.65	56.00
mean	531.42	1617.9	89.5	58.4	2.28	16.32	22.1	3.6	24.91	78.41	11.76	3.25	56.51	56.09
STD	62.17	158.71	11.09	98.64	2.06	0.9	0.84	1.41	1.6	4.46	5.4	0.55	5.32	1.66

and found that mucilage content increased significantly with applied various compost tea and/or NPK compared to control treatment.

Mucilage yield in irrigation treatments (factor A) did not show significant difference, but average of factor interaction, in four repeats showed the highest grain, biological and mucilage yield were related to conditions a_1b_3 and then a_b4 and minimum of biological, grain and mucilage yield were related to conditions a_3b_1 and then a_2b_1 . Grain, biological and mucilage yield were highest when we used Zn in concentration of 3 ppt and then Mn in concentration of 4 ppt. These results are in agreement with Bannayan *et al.*⁴, that isabgol seed yield was lower for all of the water deficit treatments compared to control. One thousand grain weight was relatively stable across all irrigation treatments, but no reduction in mucilage percentage across all water deficit treatments was observed.

The traits correlation showed that grain yield had significant positive correlation to biological yield, mucilage yield, number of spikes per plant and number of grains per plant. The biological yield had significant positive correlation to grain yield, mucilage yield and number of spikes per plant and mucilage yield only had significant positive correlation with grain yield and biological yield (Table 5). In order to select traits which have high effect on grain yield, biological yield and mucilage yield, multiple regression and stepwise regression were performed. The dependent variable was grain yield (y_1), biological yield (y_2) and

mucilage yield (y_3). Multiple correlation of grain yield was 0.957 and stepwise regression of y_1 showed number of spikes per plant was the first variable entered to model and contributed 37.6% of y_1 variation. Then 1000-grain weight was second variable entered to model and overall number of spikes per plant explained 54.4% of variation and in step 3 harvest index entered to model explained overall 75.4% of variation. In the correlation analysis number of spikes per plant also had significant and positive correlation with grain yield. Multiple correlation of y_2 (biological yield) was 0.984 and stepwise regression for y_2 showed number of spikes per plant, 1000-grain weight and harvest index entered in model, respectively, and contributed overall 85.8% of y_2 variation. In the correlation analysis, number of spikes per plant also had significant positive correlation with biological yield (Table 5). The multiple correlation for y_3 (mucilage yield) was 0.999 and in stepwise regression, harvest index, number of leaves per plant and number of grain per plant were entered to model respectively and explained overall 0.999 of y_3 variation (Table 6). In the correlation analysis, mucilage yield only had significant positive correlation with grain yield and biological yield and with harvest index ($r = 0.3$), number of leaves per plant ($r = 0.28$), and number of grain per plant ($r = 0.35$) had median correlation but it was not significant. Omidbaigi and Mohebbi¹⁰ carried out stepwise regression for seed swelling in isabgol as a dependent variable. They found only the seed yield traits other independent variable with $r^2 = 0.754$ showed a significant positive effect on seed swelling.

Table 5. Correlation coefficients of traits in isabgol under limited irrigation and micronutrient application.

	X ₁	X ₂	X ₃	X ₄	X ₅	X ₆	X ₇	X ₈	X ₉	X ₁₀	X ₁₁	X ₁₂	X ₁₃	X ₁₄
X ₁	1													
X ₂	0.996**	1												
X ₃	0.858**	0.887**	1											
X ₄	-0.135	-0.024	0.36	1										
X ₅	-0.101	0.012	0.39	0.99**	1									
X ₆	0.145	0.0507	-0.25	-0.92**	-0.91**	1								
X ₇	0.407	0.31	0.06	-0.51*	-0.51	0.343	1							
X ₈	-0.128	-0.199	0.36	0.99**	0.99**	-0.93**	-0.46	1						
X ₉	0.61*	0.58*	0.23	-0.68	-0.66**	0.65**	0.73**	-0.6**	1					
X ₁₀	0.517*	0.465	0.35	-0.396	-0.38	0.522*	0.37	-0.4	0.55*	1				
X ₁₁	-0.156	-0.05	0.34	0.99**	0.99**	-0.9**	-0.53*	0.99**	-0.7*	-0.37	1			
X ₁₂	-0.20	-0.07	0.28	0.96**	0.96**	-0.89**	-0.50	0.95**	-0.6**	0.36	0.95**	1		
X ₁₃	-0.233	-0.229	-0.33	0.219	-0.22	0.12	0.26	-0.16	0.162	0.13	-0.23	-0.08	1	
X ₁₄	-0.22	-0.08	0.13	0.67**	0.67**	-0.57*	-0.78**	0.64**	-0.6*	-0.6*	0.66**	0.63*	-0.2	1

* and ** significant at 5% and 1% levels, respectively.

Table 6. Multiple regression and stepwise regression of traits in isabgol.

	Step1	Step2	Step3
Y ₁	X ₆ *	X ₂ **	X ₁ **
r=0.957 ^a	r ² =0.376 ^b	r ² =0.544	r ² =0.754
Y ₂	X ₆ *	X ₂ **	X ₁ **
r=0.984	r ² =0.344	r ² =0.633	r ² =0.858
Y ₃	X ₁ **	X ₁₀ **	X ₇ **
r=0.999	r ² =0.998	r ² =0.9995	r ² =0.9997

a = multiple correlation coefficient, b=contribution of the parameter to the coefficient of determination (r²), * and ** significant at the level 0.05 and 0.01, respectively (X₁= harvest index, X₂= 1000-grain weight, X₃= mucilage, X₄= plant height, X₅= spike height, X₆= number of spikes per plant, X₇= number of grains per plant, X₈= swell index, X₉= number of forks in each plant, X₁₀= number of leaves in each plant, X₁₁= number of days from flowering to maturity).

In order to select traits which have the highest effect on mucilage yield, we have performed path analysis. According to correlation analysis, grain yield and biological yield had direct effect to mucilage yield. Correlation analysis and stepwise regression traced diagram of mucilage yield and distinguished traits which have direct and indirect effect (Fig. 1). Direct effect content of grain yield and biological yield to mucilage yield was 0.705 and 0.305, respectively. Grain yield had highest effect to mucilage yield. Indirect effect of number of grain per plant, number of spikes per plant and plant height also were -0.00909, 0.0777 and 0.01979, respectively. Number of spikes per plant via influence of grain yield and biological yield had highest indirect effect on mucilage yield. Therefore, in order to increase mucilage yield it is favorable to select plants according to grain yield and number of spikes per plant (these traits must have high inheritance). Content of number of spikes per plant was high in condition of common irrigation and use of Zn in concentration of 3 ppt.

PCA analysis for mean of factors interaction (A and B) showed 3 PCs with eigenvalues higher than 1 accounted for 88% of the total variation. PC₁, PC₂ and PC₃ explained 54.6%, 24.8% and 0.08% of the total variation respectively (Table 7). The correlation of

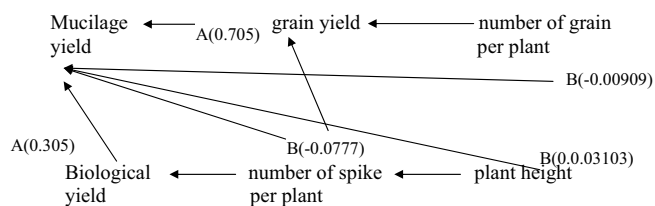


Figure 1. Diagram of direct and indirect effects in isabgol.

A = direct effects, B = indirect effects.

PC₁ and PC₂ with traits showed PC₁ had significant and positive correlation with harvest index, 1000-grain weight, spike height, swell index, number of forks per plant, number of days from flowering to maturity and PC₂ with harvest index, 1000-grain weight, spike height, swell index, number of forks per plant, number of days from flowering to maturity at the 0.01 level (Table 8). Chart of biplot based on PC1 and PC₂ and chart of density of 3D plot showed a₁b₃, a₁b₄ and a₁b₅ having high content of PC₂ were located in a separate group, a₁b₂ which had high content of PC₁ was located in a separate group and the rest of treatment that has medium content of PC₁ and PC₂ was located in separate group (Figs 2 and 3).

Table 7. Eigenvalues and proportion of total variability explained by the first three principal components (PC).

Principle component	Eigenvalue	Variance %	cumulative
PC1	7.64	0.546	0.546
PC2	3.43	0.248	0.794
PC3	1.22	0.087	0.881

Table 8. Correlation of PC_s with traits.

Traits/ PC	PC ₁	PC ₂
X ₁	-0.305	0.926**
X ₂	-0.192	0.949**
X ₃	0.185	0.969**
X ₄	0.966**	0.2
X ₅	0.96**	0.234
X ₆	-0.904**	-0.133
X ₇	-0.66**	0.296
X ₈	0.957**	0.205
X ₉	-0.814**	0.427
X ₁₀	-0.557*	0.464
X ₁₁	0.964**	0.182
X ₁₂	0.938**	1.131
X ₁₃	-0.237	-0.303
X ₁₄	0.783**	-0.046

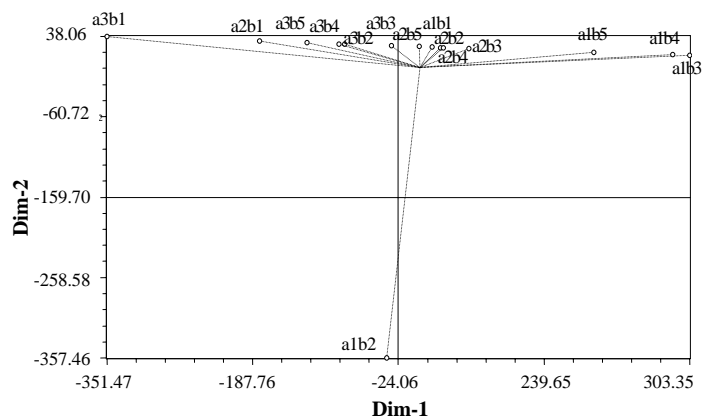


Figure 2. Biplot of PC₁ and PC₂ in isabgol under limited irrigation and using micronutrient.

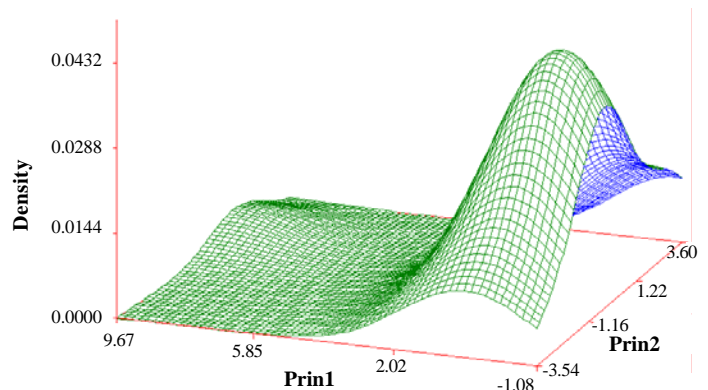


Figure 3. Density plot of principle component analysis of isabgol for traits x₁-x₁₄.

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