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**THE STUDY EFFECT OF BRASSINOSTEROID APPLICATION ON YIELD AND  
YIED COMPONENTS OF COWPEA (*VIGNA UNGUICULATA*) UNDER WATER  
STRESS CONDITIONS**

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**ABSTRACT**

For study effect of water stress and Brassinosteroid application on cowpea yield and growth, Kamran Cultivar. Experiment was performance at research educational farm of Islamic azad university, Imam Khameini Yadegar Rey Branch located in south of Tehran in 2013. The experiment was a split plot in a randomized complete block design with three replications. Main factor include two irrigation levels.

CIO: irrigation after 60 Millimeter evaporation from evaporation washbasin class A as normal condition, it, irrigation after 120 millimeter evaporation of evaporation washbasin class A as draught stress conditions). Sub factor also include as five Brassinosteroid application levels by different densities B0: not using of Brassinosteroid (bating seeds on distilled water). B1: bating seeds on Brassinosteroid with 2 Micro molar density; B2: bating seeds on Brassinosteroid with 4 micromolar density, B3: Brassinosteroid leaf-spray with 2 micro molar density, B4: Brassinosteroid leaf spray wit 4 micromolar density : results indicate that draught stress reduce characteristics like sheath number on bush (16 percent) , seed number on sheath (9 percent) thousand seeds weight (23 percent) seed yield ( 21 percent ) , biologic yield (35 percent) . Draught stress didn't have meaningful effect on harvest index, using of Brassinosteroid hormone effect on all studied characteristics.

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Brassinosteroid effect on all yield components toward decreasing draught stress adverse effects and finally increase meaningfully seed yield. This it suggest that for decreasing adverse effects of draught stress in cowpea, we can use Brassinosteroid as leaf spray with 2 micromolar density or as bating seeds with 4 micromolar density by considering this research results.

**Keywords: Brassinosteroids, Drought Stress, Yield, Cowpea**

## **INTRODUCTION**

Population growth and country social and economic development cause to increase consumption of protein materials especially red meat in two recent decade , in this basis , production increase of protein material especially plant proteins that are valuable resources in feeding , is ineviatable. (Parsa & Bagheri, 1387). Bean seed account as one of important plant resourses rich of protein after cereals and second important human food resource. ( parsa&bagheri 1387) amany beans , cowpea (*Vigna unguiculata*) contain 21/32 percent protein , 2/82 percent fat and 60/3 percent carbohydrate : also , it is rich resource of calcium and ferrous. (Ghorbani et al., 1384). That yield well in soil tissues, from heavy clay to sandy, in vast range. (Valenzoela and smith , 2002). About 50% percent of different products agriculture in iran affected because of water shortage and raining inappropriate distribution. (Vaezi & Ahmadikhah, 1389). Plants response to this stress or become compatible with it by change in cellular metabolism and defense

mechanisms induction. (bahnert et al 1995). Draught stress effect on agricultural plant growth, tillering, leaf photosynthesis leaf sense cence, seed number, seed size. (Fredrik and combert, 1995). anyia et al report that draught cause biomass reduction in to genatype of cowpea. Water shortage in different growth stage limit plant physiologic activities till forming and filling of seed stage and decrease seed yield. (Gau et al, 2007). Decrease of seed yield due to draught stress reported by falahatzadeh (1386) on wheat, kazeminasab (1384) on corn, pour taghi (1389) on visor and baghaei (1383) on bean. Kazemid et al (1387) found bean seed yield under normal irrigation condition is more than draught stress, and see yield reduction in draught stress treatment accur due to yield components reduction. (nadali et al , 1389 ) use of plant growth regulator materials such Brassinosteroids become pratical approach Brassinosteroids are plant esteriod gradients with wide biological activity , thet able to increase plants yield by plant metabolism

changes and their protection against environment stresses . (Eskandari, 1389) Brassinosteroids cause to increase plants resistance against non-living different stresses. These materials in molecular level cause to changer gene expression and metabolism change and protein and nucleic acid biosynthesis (Khripachetal, 1999). Zhang et al (2008) reported that Brassinosteroid application treatment increased activity aome anti-oxidant enzymes and cause decreasing oxidative stress. Brassinosteroid application also reported in increasing resistance to draught in sugar-beet, such that use of Brassinosteroid remedy completely reduction in biomass that made by draught stress. (Schilling et al,1997). Brassinosteroid external application in rice cause to improve growth, increase of prolin and protein and anti-oxidant anzyme activity and use of this hormone cause to increase different gene expression related with oxidative stress. (sharma et al , 2013). Use of Brassinosteroid at draught stress conditions improved wheat growth. (shabaz et al , 2008 ) , green bean (Upreti and Murti,2004) and Tamato

(Ogweno et al 2008).so , it appear necessary to determine appropriate and effective density of Brassinosteroid in different growth stages of cowpea that reduse draught adverse impacts , effectively.

**MATERIAL AND METHODS**

Situation and place of experiment conduct this research performed in research educational farm of Islamic azad university , imam Khomeini yadegar rey Branch locatade in south of Tehran in 2013. Farm height of sea level is 1000 meter and farm located in geographical width 35 degree and geographical length 51 degree and 29 minute in view of geographical situation. And it's average year raining is about 216 millimeter. It's year temperature average reach to 17/1 c.

**Soil analysis**

Before performing experiment sample from soil for measuring soil physical and chemical characteristics of experiment place. Several sampling performed of 0 – 30 cm depth for this act and sent to laboratory, table (1- 3) indicate soil chemical and physical characteristics.

**Table 3.1 Test results of soil experiments**

send	silt	clay	k	p	OC	Total N	T.N.V	pH	EC	Depth
%	%	%	ppm	ppm	%	%	%		«Ds/m»	cm
18	40	42	503.2	19.6	1.13	0.11	13.65	8.05	3.58	0-30
					B	Mg	Cu	Zn	Fe	Soil tissue
					ppm	ppm	ppm	ppm	ppm	
					2.98	3.28	1.4	0.6	2.8	clay

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**Test plan and treatments**

This experiment performed as comminuted crete in highly random blocks frame by three repeat. Main factor include two irrigation levels (IO: irrigation after 60 millimeter evaporation from evaporation washbasin class A as normal condition IT: irrigation after 1020 millimeter evaporation of evaporation washbasin class A as draught stress condition). Sub factor also include five Brassinosteroid application levels by different densities (BO: not using of Brassinosteroid (bating seeds on distilled water), B1: bating seeds on Brassinosteroid with 2 micromolar density, B2: bating seeds on Brassinosteroid with 4 micromolar density B3: Brassinosteroid leaf-spray with 2 micromolar density B4: Brassinosteroid leaf-spray with 4 micromolar density. Any crete include four plant line with 4 meter length by 50 cm distance and distance between bushes was 10 cm on any plant line between repeat that 3 meter distance was considered two creeks located in this distance. One considered for lower repeat irrigation and other considered as higher repeat drain. Distance of two sub crete from each other considered one meter (two non-plant stack) and distance of two main cretes from each other considered two meter (4 non-plant stack) for decreasing edge

impacts and maintain growth uniformity and sampling.

**Irrigation**

First irrigation immediately after plant, later stages of irrigation performed till pruning based on plant need and environmental conditions. And after that, irrigation treatments after 60 millimeter evaporation of evaporation washbasin and irrigation after 1020 molimeter evaporation of evaporation washbasin. (respectively as desired conditions and draught stress).

**Brassinosteroid solution spray**

Different cretes solution spray with Brassinosteroid performed two time according to plan in growth stage (6 leaf) and budding. Solution spray performed in early morning and sunrise time. Handy sprayer machine with 10 liter volume used for solution spray. Yield and seed yield components 5 bush of second line any crete harvest randomly after physiological examination and simultaneous by final harvest. And sheath number in bush, seed number in sheath, thousand seeds weight and bush height were measured their final harvest performed in first week of mehr month, 2013 after drying and browning leaves, sheaths and also seeds hardening.

Final harvest occur of level equivalent to 1.5 m<sup>2</sup> (from third line of any crete by removing 0.5 m from first and end of any line). All harvested bushes, after separating seeds of sheaths and drying seeds before sun determined seed yield. Then, other parts of bushes were placed in ovan with temperature 75<sup>0</sup>C for 48 hours, till dry quietly and yield air body dry weight (except seeds). Harvest index result by splitting seed yield on biomass (dry weight of bushes plus seed yield).

### Statistical analysis

Data analyze using MSTATC software. Donken test used for comparing average in 5% percent level. Determination of coefficient rate between characteristics done using of SPSS software. Tables and diagrams draw by EXCELL 2007 software.

### Results and discussion

#### The number of bush sheath

The results of data variance analysis indicate that all tested treatments had meaningful effect on bush (table 2) draught stress cause to decrease bush sheath number and lower it 16 percent. Bush sheath number increased 27 percent toward control treatment due to Brassinosrerioid application as leaf spray with 2 micromolar density. Bush sheath anumber increased meaning fully by increase Brassinosrerioid density at seed bating mode.

But bush sheath number decreased by increased density at leafspray mode. highest bush sheath number yield of draught non\_stress treatment and brassina steroid leaf spray with 2 micro molar density by average 17/20 lowest bush sheath number also yield of draught stress treatment with non\_application of Brassinosrerioid by average 11/23 .(table 4/0 acosta-gollegoset et al(1991) reported that produced sheathes in any bush decreased in water shortage stress dueto decreasing of flower bush number and also flower falling kazemi et al (1387) mentioned disorder in pollination act from decrease results of sheath number in drought stress conditions. Also, Brassinosrerioid effect on changes in bean nodulation (phaseollusvulgaring reported by uperti and muti (2004). Theyfoundthat Brassinosrerioid improved root naulation and sheath yield in irrigated and stressed plants.

#### Seed number in sheath

Results of data verianse analysis indicate that all tested treatments had meaningful effect on seed number at sheath (table2). Draught stress decreased 9 percent seed number in sheath toward normal irrigation, but Brassinosrerioid application as leafspraywith 2, micromolar density could increase 18 percent seed number in sheath. Toward control treatment. (table3). Totally, highest seed number in

sheath yield of draught non-stress treatment and Brassinosrerioid leafspray with 2 micromolar density by average 7/79 and its lowest number yield of draught stress treatment and bating seed in Brassinosrerioid with 2 micromolar densities by average 5/83 that of course, this treatment didn't have meaningful difference with Brassinosrerioid non-application (table 4).

Nan-prauiding of necessary photosynthesis material for embryo growth and seed evolution is one of important reasons in dereasing seed number on sheath and bush at draught stress conditions (goldani&rezvanimoghadam, 1386).Decreasing of seed number in sheath of bean reported by other researchers. (monem, 1388 , rezaee and haghghi , 1388). Shahid et al (2011) in study on pea, mentioned that seed number in sheath under Brassinosrerioid increased 26 percent toward control treatment.

Results of data varians analysis indicate that all tested treatments had meaningful effect on thousand seeds weight (table 2) thousand seeds weight decreased 23 percent under draught stress and reached of 188/89 gr in normal irrigation condition to 145/43 gr in draught stress condition Brassinosrerioid cause meaningful increase of thousand seeds and

seed bating in Brassinosrerioid with 4 micromolar density increased 68 percent thousand seeds weight toward control treatment (table 3). Use of Brassinosrerioid in draught stress condition could remove well stress effect and don't decrease thousand seeds weight such that , thousand seeds weight in all Brassinosrerioid application in draught stress conditions was equivalent or more of Brassinosrerioid non-application mode in normal humid condition (draught non-stress). Totally, highest thousand seeds weight obtained of draught non-stress treatment associated with seed bating in Brassinosrerioid with 4 micromolar density by average 246/4 gr .lowest thousand seeds weight also yield of draught stress treatment and Brassinosrerioid non-application by average 109/5 gr . ( table 4 ). Girk et al (2006) reported that seeds weight decreased in draught stress due to photosynthesis material reduction and reduction of material transfer toward seed and also decreasing of seed filling period length and accelerating in riping. shahid et al. (2011) expressed that thousand seeds weight of pea increased 18 percent under Brassinosrerioidapplication toward control treatment these researcher expressed that Brassinosrerioid cause to

increase food transfer to seed by improving plant growth

### Seed yield

Results of data variance analysis indicate that all tested treatments had meaningful effect on seed yield. (table2). Seed yield decreased 41 percent under draught stress and of 4180/8 kg/hectar in draught non-stress conditions reached to 2451/8 kg/hectar under draught stress condition.

But, use of Brassinosrerioid could increase meaningful seed yield and seed yield increased 3times in best mode toward control treatment. (table3) use of Brassinosrerioid under draught stress condition could prevent of decreasing seed yield due to drought stress. Such that all Brassinosrerioid application treatments draught stress condition produce equivalent or more seed yield than Brassinosrerioid non-application and draught non-stress treatment .totally , treatment yield yield of draught non-stress treatment associated with bating seed in Brassinosrerioid with 4 micromolar density by average 5899 kg/hectar. Lowest seed yield amount also relate to draught stress treatment and Brassinosrerioid non-application by average 1349 kg/hectar. (table4). Seed yield decrease under draught stress could decrease yield components like sheath number in bush, seed

number in sheath and thousand seed weight (nadali et al 1389). liebman et al (1995) expressed yield component in bean include sheath number in bush seed number in sheath , hundred seed weight , that these factors had important role in determining bean yield , also , seed yield increase with Brassinosrerioid application reported in plants such wheat ( Sairam , 1994) and india mustard (Ali et al , 2006 ).

### Biologic yield

Results of data variance analysis indicate that draught stress and Brassinosrerioid hormone application had meaningful effect on biologic yield but their counter effect on biologic yield wasn't meaningful (table 2). Biologic yield decreased 35 percent due to draught stress and of 10705/6 kg/hectar under draught non-stress reached to 6933/2 kg/hectar in stress condition (table 3). Biologic yield increased with Brassinosrerioid application. Of course, Brassinosrerioid application as seed bating with 2 micromolar density by average 7319 kg/hectar not only didn't have meaningful effect on biologic yield increase, but also produce lowest biologic yield rate. That of course, this treatment produce lowest biologic yield rate. That of course, this treatment didn't have meaningful difference with Brassinosrerioid non-application treatment. But , increase of

biologic yield was meaningful in other Brassinosrerioid application treatment , and highest biologic yield gain of Brassinosrerioid leaf spray treatment with 2 micromolar density by average 104050 kg/hect ( table 3) results of Anyia et al ( 2004) study about dried matter accumulation , indicate that bush dried weight decreased under draught stress condition due to photosynthesis and also plant flower early for escaping of draught and survival then we can tell that stress cause to accelerate growth and to reduce plant growth period .

They also report that draught cause to decrease biomass in studied 10 cowpea genotypes .also , increase of plant dry weight by Brassinosrerioid application verified before by some researchers .( Ali et al , 2006 ; Sairam , 1994 )

### **Harvest index**

Results of data variance analysis indicate that draught stress didn't have meaningful effect on harvest index but Brassinosrerioid hormone application and counter effect of draught stress and Brassinosrerioid hormone was meaningful on harvest index .( table 2 ). By considering that drought stress decreased equally biologic yield and seed yield, thus, harvest index didn't subject draught stress. Use of Brassinosrerioid had meaningful effect

on harvest index and increased it 22% percent. (table3) We can tell by considering results that Brassinosrerioid effect on seed yield especially in draught stress condition cause harvest index didn't decrease very much in draught stress condition .totally , highest harvest index gained from draught non-stress treatment associated seed bating in Brassinosrerioid with 4 micromolar density by average 51/14 percent . Lowest harvest index yield of Brassinosrerioid non-application treatment respectively by average 21/77 and 25/32 percent both in draught stress condition and both in draught non-stress condition (table 4). By consider in that Brassinosrerioid increase seed yield and biologic yield , it appear that increase of harvest index under Brassinosrerioid hormone was due to more increase of seed yield toward biologic yield. Increase of harvesr index under Brassinosrerioid application also reported in sesame (Karnatake, 2008). Researches also at study on sorghum expressed that Brassinosrerioid cause to increase harvest index.

### **CONCLUSION**

This research results indicate that draught stress decreased characteristics like bush sheath number , seed number in sheath , thousand seeds weight , seed yield and



biologic yield . Use of Brassinosreriod subject all studied characteristic. Brassinosreriod subject all studied index toward decrease of draught stress average effects, and finally cause to increase meaningful seed yield. in majority of characteristics , Brassinosreriod leaf-spray effectiveness with 2 micromolar density was more , but in cause of seed yield , seed bating in Brassinosreriod with 4

micromolar density prudence more seed yield that of course didn't have meaningful difference with Brassinosreriod leaf spray treatment with 2 micromolar density . it suggest by considering this research results that for decreasing draught stress adverse effects in cowpea can use of Brassinosreriod as leaf spray with 2 micromolar density are as seed bating with 4 micromolar density.

**Table 2. Analysis of variance for number of pods per plant, number of seeds per pod, seed weight, seed yield, biological yield and harvest index under drought conditions, and the use of cowpea Brassinosreriod**

Harvest index	Mean squire					Degrees of freedom	Source changes
	Biological Yield	Seed Yield	Thousand seed weight	Number of seeds per pod	Number of pods per plant		
26.101 <sup>ns</sup>	3382941.9 <sup>**</sup>	78765.633 <sup>ns</sup>	319.319 <sup>ns</sup>	0.0001 <sup>ns</sup>	0.775 <sup>ns</sup>	2	Replication
73.078 <sup>ns</sup>	106732513.2 <sup>**</sup>	22627030.5 <sup>**</sup>	14164.049 <sup>**</sup>	3.367 <sup>**</sup>	52.272 <sup>*</sup>	1	Drought stress
15.204	15197.7	88371.23	24.635	0.003	0.793	2	Main error
403.167 <sup>**</sup>	9951441.8 <sup>**</sup>	6977405.91 <sup>**</sup>	5623.346 <sup>**</sup>	1.226 <sup>**</sup>	11.162 <sup>**</sup>	4	Brassinosreriod × Drought stress
64.472 <sup>**</sup>	1044848.86 <sup>ns</sup>	806315.78 <sup>**</sup>	644.468 <sup>**</sup>	0.223 <sup>**</sup>	0.318 <sup>*</sup>	4	Brassinosreriod
12.350	363278.383	74289.6	112.107	0.032	0.086	16	Minor error
9.5	6.83	8.21	6.33	2.73	2.02	-----	Coefficient of (%) Variation

ns :<sup>\*</sup>and<sup>\*\*</sup> :To the non-significant and significant at 5% and 1%.

**Table 3. Comparison of the effects of drought and Brassinosreriod use of steroids on the number of pods per plant, number of seeds per pod, seed weight, seed yield, biological yield and harvest index of cowpea**

Harvest index (%)	Biological Yield (kg.hec)	Seed Yield (kg.hec)	Thousand seed weight (gr)	Number of seeds per pod	Number of pods per plant	Treatment
38.56	a	10705.6	a	4180.8	a	Irrigation
35.43	a	6933.2	b	2451.87	b	Non drought stress
23.54	c	7768	c	1840	d	Drought stress
38.36	b	7319	c	2771	c	Brassinosreriod Control
45.68	a	9595	b	4476	a	2micro molar soaking
40.23	b	10450	a	4226	a	4micro molar soaking
3716	b	8968	b	3290	b	2micro molar sprayed leaves
				175.5	b	4micro molar sprayed leaves
				169.2	bc	

Each column of numbers in each group with the same letters, no statistically significant differences according to Duncan's test at the 5% level, respectively.

**Table 4. Comparison of the interactions between stress and the use of steroids Brassinosreriod of pods per plant, number of seeds per pod, seed weight, seed yield, biological yield and harvest index of cowpea**

Harvest index (%)	Biological Yield (kg.hec)		Seed Yield (kg.hec)		Thousand seed weight (gr)		Number of seeds per pod		Number of pods per plant		Treatment		
	d	b	e	d	d	d	d	d	d	d	Brassinosreriod	Irrigation	
25.32	d	9331	b	2330	e	137.3	d	6.333	d	14.07	d	control	
36.89	c	9253	b	3408	d	171.3	c	6.797	c	15.23	c	2micro molar soaking	Non
51.14	a	11560	a	5899	a	246.4	a	7.210	b	16.37	b	4micro molar soaking	Drought
44.25	b	11900	a	5250	b	199.5	b	7.790	a	17.20	a	2micro molar sprayed leaves	stress
35.18	c	11480	a	4057	c	189.9	b	6.543	cd	16.23	b	4micro molar sprayed leaves	
21.77	d	6205	de	1349	f	109.5	e	5.913	e	11.23	g	control	
39.83	bc	5384	e	2133	e	147.3	d	5.830	e	12.10	f	2micro molar soaking	
40.23	bc	7627	c	3054	d	170.2	c	6.420	d	14.23	d	4micro molar soaking	Drought
36.21	c	8997	b	3201	d	151.6	d	6.747	c	15.03	c	2micro molar sprayed leaves	stress
39.14	bc	6454	d	2523	e	148.5	d	6.413	d	13.30	e	4micro molar sprayed leaves	

Each column of numbers in each group with the same letters, no significant differences according to Duncan test at the 5% level, respectively.

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