2011 2nd International Conference on Agricultural and Animal Science
IPCBEE vol.22 (2011) © (2011) IACSIT Press, Singapore

Biological Parameters of Cottonseed (*Gossypium hirsutum* L. N200 Cultivar) as Affected by Irrigation Regimes and Foliar Application of Micronutrients

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Abstract. Cotton, which is grown for using fiber and oil, is one of the most important and strategic crops through the world especially in Iran. First study has been performed to evaluate effects of different soil moisture status and foliar application of micronutrients on quantitative and qualitative traits of cotton at agricultural researches station belongs to the Islamic Azad University, Khorasgan Branch (Isfahan), in a split plot layout with three replications in 2009. Farm irrigation treatments namely, irrigation after 80 and 160 mm evaporation from evaporation pan class "A" were set as the main factors and foliar application of micronutrients i.e. control (pure water spray), two times and three times foliar application of micronutrients were set as sub-factors. To assess influence of done treatments during growing season of female parents on cottonseed germination parameters, seed from F1 generation replanted in both farm and laboratory in the following year (2010) and a lot of correlated tests have done. According to the results, irrigation after 80mm evaporation increased seed oil percentage compare to irrigation after 160mm evaporation up to 16%. Though, the opposite result has shown about seed protein percentage by which irrigation after 160mm evaporation improved this trait over the irrigation after 80mm evaporation treatment by 17%. Irrigating treatments during female parents' growing season had significant effect on percentage and rate of germination, seedling fresh and dry weight, and shoot length and seed vigor. For instance, in farm germination test, irrigation after 80 mm evaporation increased germination percentage and rate of germination by 14 and 17%, respectively. Almost, the same results observed about foliar application of micronutrients. The highest germination percentage (91.67%) and rate of germination (2.115) were linked to three times foliar application of micronutrients; meanwhile, the lowest amounts equal to 84.17% and 1.755 were related to control. Interaction of irrigation regimes and foliar application of micronutrients significantly affected just rate of germination at 5% probability level in the section of farm germination test. The highest rate of germination (2.267) was related to irrigation after 80 mm evaporation with three time foliar application of micronutrients, although the lowest amount (1.520) was recorded in irrigation after 160 mm evaporation in control.

Keywords: Cotton, Germination, Irrigation regime, Seed viability, Tetrazolium test

1. Introduction

Cotton, the king of fiber crop is the most important commercial crop in the world. To increase the cotton production in the country, it is necessary to increase cultivation of hybrid cotton under irrigated areas and for this, supply of good quality seed is essential. It has been already demonstrated that cotton productivity can be increased to an extent of 20 per cent, merely by using quality seed. Apart from major nutrients, micronutrients also play a vital role in seed production (Anonymous, 1995). The dire need for intensive land use drew attention for applying micronutrients to cotton. Essential micronutrients like Zinc, Iron, Manganese, Copper, Boron and Magnesium play an important role in physiology of cotton crop and these are being a part of enzyme system or catalyst in enzymatic reactions. They are required for plant activities such as aspiration, meristamatic development, chlorophyll formation, photosynthesis, energy system, protein and oil synthesis, gossypol, tannin and phenolic compounds development (Anonymous, 1995). Certain micronutrients may

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help to secure uniform emergence, rapid seedling growth and healthy plant stand. Some beneficial effects on seed yield and quality as reflected in viability may be achieved by applying micronutrients. Effects of foliar application of micronutrients on cotton yield and fiber quality have been widely studied (Karev, 1980; Khuzhanazarov *et al.*, 1983). Verma *et al.* (1973) observed that, one ppm boron significantly increased the fresh and dry weight of root (28.5 g and 6.25 g, respectively) in tomato crop. Harb (1992) reported that, soaking the cotton seeds in micronutrients (manganese, zinc and boron) at 50 ppm showed significant influence on shoot and root length. The highest increase in fresh weight and dry weight of seedling were recorded with combination of manganese, zinc and boron at 100 ppm. Rathinavel *et al.* (2000) reported that foliar application of zinc sulphate (0.5%) on 90 and 110 days after sowing increased 100 seed weight, germination, speed of germination, root length, shoot length and vigor index. The values for the electrical conductivity of seed leach ate did not show consistency for treatments. Ullagaddi (2000) observed significantly higher germination, shoot length, root length and vigor index were observed with foliar spray of ZnSO4 (0.1%) plus boron (0.1%) combination with GA3 (50 ppm). There is a positive correlation between irrigation time and nutritional status of plant. Crops which uptake proper and adequate amounts of nutritious elements show more effective resistance against water stress. In other words, water use efficiency in such plant is higher. It means that by using particular amount of water, plant productivity will be improved (Anter *et al.*, 1978). An appropriate seed establishment in farm commences with well-developed seeds (Acquaah, 2002). Seed filling conditions on female parents, environmental stresses and storing conditions impress qualitative traits of seed that may affect seed germination and seedling establishment and lead to change in farm uniformity, production, cultural and management costs' and finally, crops yield (Viera *et al.,* 1992). Seed germination stage is one of the most critical stages for seedling establishment which is a determinative factor in order to have efficient production. Seedling establishment in farm is the result of interaction of environmental factors and seed's biological characteristics. Khaje-hoseini *et al.* (2003) reported that water stress in seed filling period has decreased soybean yield but, had no significant effect on germination percent and seed vigour. They pointed out that water stress during this crucial period can't directly change metabolic activities in seeds related to seed's biological parameters.

2. Materials and methods

The first study conducted at agricultural researches station owned by Islamic Azad University, Khorasgan Branch in 2009. The experimental design was a split plat layout based on randomized complete block design with three replications. Farm irrigation treatments such as, irrigation after 80 and 160 mm evaporation from evaporation pan class "A" were set as the main factors and foliar application of micronutrients i.e. control (pure water spray), two times foliar application of micronutrients (early flowering and one week later) and three times foliar application of micronutrients (Early flowering, one and two weeks later) were set as sub-factors. The source of fertilizer was complete chelation of metallic micronutrients by EDTA ensure rapid uptake and protection from premature fixation contains 4% Zinc, 4% Iron, 3% Manganese, 0.5% Copper, 1.5% Boron, 0.05% Molybdenum, 2.2% Magnesium oxide, 1.3% Magnesium and 1.3% Sulfur. Seeds were sown on May 13th with 8.8 plants per square meter density. First irrigation was done right away after planting seeds. Phonological, morphological, yield, fiber qualitative and cottonseed traits were recorded and calculated.

Second section carried out simultaneously as farm and laboratory researches in 2010. The same cultural operations and experimental layout were performed as well, with this difference that in farm and laboratory germination test, seeds of F1 generation (earned from 2009 study) were used. A sample of four hundred seeds were drawn from each treatment at random and sown at a distance of 20 cm between the rows and 10 cm between the seeds in four replications on a well prepared seed bed. The emergence count was made on 12th day after sowing. The newly germinated seeds with two centimeters growth above the soil surface were considered for recording field emergence and were expressed in percentage. In farm germination test, we evaluated just biological traits such as percentage and rate of germination. Although in the laboratory section we studied percentage and rate of germination, seed vigour (by tetrazolium test), seedling fresh and dry weight, length of root and shoot according to the Association of Official Seed Analysts (1988) to evaluate effects of irrigation regims and foliar application of micronutrients during female parents growing season on seeds' biological parameters. Before each test seeds were surface sterilized by using ten per cent hypochlorite solution and fungicide at the rate of two per thousand. For laboratory percent of germination test, hundred seeds were taken at random from each treatment and uniformly placed on wet germination paper in four replications (25 seeds per each replication). The wet germination papers were kept in the seed germinator named Jacobsen maintained at constant temperature of 25^{oc} and 90% relative humidity. The first and final counts were taken on 4th and 12th day, respectively. The numbers of normal seedlings were counted and the means of four replications expressed as germination percentage (Okcu *et al*., 2005). To measure rate of germination, same test has done and finally by using related equation rate of germination recorded. From the germination test, ten seedlings were selected randomly from each treatment in replication wise on 12th day. The shoot length was measured from the base of the primary leaf to the base of hypocotyls and the mean shoot length was expressed in centimeters. Ten normal seedlings used for shoot length measurement, were also used for the measurement of root length. It was measured from tip of primary root to the base of hypocotyls and mean root length was expressed in centimeters. The same ten normal seedlings selected for shoot and root length measurements were weighted as seedling fresh weight. After that were taken in paper packet and were kept in an oven maintained at 70^{oc} for 24 hours. After drying, the seedlings were kept in desiccators for cooling. The weights of dried seedlings were recorded and mean weight was calculated per seedling and was expressed in milligrams (Anonymous, 1996). In order to measure seed vigour, four hundred seeds were taken at random from each treatment (100 seeds in four replications) sterilized and soaked at the 30^{oc} for 40 hours. Then seed coats were removed and cotyledons were transformed to 1% tetrazolium solution with neutral PH (about 7) at 30^{oc} and were kept in a murky place. After this time, live seeds were counted and means of four replications reported as seed vigour (Agrawal and Dadlini, 1992). The data were analyzed with MSTATC software and means comparison were performed with Duncan Test at the 5% probability level.

3. Results and discussion

Seed is the most critical and basic input for attaining higher productivity in various crops. The crucial importance of the seed has been recognized from time immemorial and attempts were made for identification and development of a proper seed, which holds the key for agricultural abundance. Thus, the seed lies at the root of our planning for prosperity. As can be seen, effect of irrigation regimes on germination percentage and rate of germination in farm germination test was significant at 1% probability level (Table 3). Nevertheless the maximum germination percentage (95.00%) and rate of germination (2.106) observed in irrigation after 80mm evaporation treatment. By contrast, minimum germination percentage (81.66%) and rate of germination (1.750) were observed in irrigation after 160mm evaporation treatment (Table 4). In other words, irrigation after 80 mm evaporation has increased germination percentage and rate of germination by 14 and 17%, respectively. Germination percentage at 5% and rate of germination at 1% probability level were affected by foliar application of micronutrients (Table 3). The maximum germination percentage (91.67%) and rate of germination (2.115) and the minimum germination percentage (84.17%) and rate of germination (1.775) were related to three times foliar application of micronutrients and control treatment, respectively (Table 4). As you can see, times foliar application of micronutrients over control has improved germination percentage and rate of germination up to 8 and 17%, respectively. Thus, may be due to better translocation and metabolism and also synthesis and accumulation of photosynthetic assimilates on the building up of efficient photosynthesis structure in the early stage of the plant cycle. Similar results were also noticed by Rubes (1984) in pea and Rathinavel *et al.* (2000) in cotton. Interaction of irrigation regimes and foliar application of micronutrients had no significant effect on germination percentage; meanwhile, affected rate of germination at 5% probability level. However, the maximum germination percentage (100%) and rate of germination (2.267) were belonged to irrigation after 80mm evaporation with times foliar application of micronutrients and minimum germination percentage (78.33%) and rate of germination (1.520) were belonged to irrigation after 160mm evaporation in control treatment (Table 4). On the basis of above, it seems that irrigation after 80mm evaporation and proper plant nutrition during grows of female parents and seed filling period had considerable effect on seeds' biological traits. Micronutrients, especially zinc and magnesium play a key role in plant's photosynthetic activities. Using such elements causes in increasing concentration of "a" and "b" chlorophyll that leads finally stimulating production of photosynthetic assimilates, increasing 100-seed weight, cottonseed yield and seed oil content.

Table 3: Analysis of variance of cottonseed germination parameters in 2010

Source of Variation	Degree of Freedom	Farm		Laboratory						
		Germination Percentage	Rate of Germination	Germination Percentage	Rate of Germination	Seedling Fresh Weight	Seedling Dry Weight	Shoot Length	Root Length	Seed Vigour
Replication	2	104.167	0.001	192.889	0.099	0.001	0.001	1.542	0.514	0.056
Irrigation	1	800.000**	0.569**	910.222*	3.672*	0.016**	0.008*	26.889**	15.125$^{N.S}$	46.722**
Ea	2	4.167	0.002	16.889	0.197	0.001	0.001	0.194	1.625	0.389
Foliar Application	2	87.500*	0.195**	32.889$^{N.S}$	0.188$^{N.S}$	0.001$^{N.S}$	0.001**	4.333**	7.264**	8.389**
Irrigation × Foliar Application	2	12.500$^{N.S}$	0.015*	32.889$^{N.S}$	0.032$^{N.S}$	0.001$^{N.S}$	0.001$^{N.S}$	0.778$^{N.S}$	0.875$^{N.S}$	0.056$^{N.S}$
Eb	8	10.417	0.002	18.222	0.048	0.001	0.001	0.722	0.340	0.306
C. V. (%)		3.65	2.26	6.12	5.41	5.19	6.45	5.15	7.17	3.24

*, ** Significant at P=0.05 and P=0.01 level, respectively. NS: Not Significant.

Table 4: Mean comparison of cottonseed germination parameters in 2010

Treatments	Farm		Laboratory						
	Germination Percentage (%)	Rate of Germination	Germination Percentage (%)	Rate of Germination	Seedling Fresh Weight (mg)	Seedling Dry Weight (mg)	Shoot Length (cm)	Root Length (cm)	Seed Vigour (%)
Irrigation									
After 80 mm evaporation	95.00 ^{a}	2.106 ^{a}	76.88 ^{a}	4.49 ^{a}	299 ^{a}	79 ^{a}	7.056 ^{a}	9.056 ^{a}	98.66 ^{a}
After 160 mm evaporation	81.66 ^{b}	1.750 ^{b}	62.66 ^{b}	3.59 ^{b}	239 ^{b}	37 ^{b}	4.611 ^{b}	7.222 ^{b}	95.45 ^{b}
Micronutrients Application									
Control	84.17 ^{b}	1.755 ^{c}	67.33 ^{a}	3.853 ^{b}	263 ^{a}	50 ^{c}	5.333 ^{b}	7.167 ^{b}	96.00 ^{c}
Two times foliar application	89.17 ^{a}	1.913 ^{b}	70.00 ^{a}	4.083 ^{ab}	270 ^{a}	56 ^{b}	5.667 ^{b}	7.917 ^{b}	96.83 ^{b}
Three times foliar application	91.67 ^{a}	2.115 ^{a}	72.00 ^{a}	4.202 ^{a}	273 ^{a}	66 ^{a}	6.500 ^{a}	9.333 ^{a}	98.33 ^{a}
Irrigation × Foliar Application									
80 mm × control	90.00 ^{b}	1.990 ^{bc}	72.00 ^{b}	4.227 ^{b}	290 ^{ab}	70 ^{a}	6.500 ^{b}	8.000 ^{b}	97.67 ^{bc}
80 mm × two times foliar application	95.00 ^{ab}	2.060 ^{b}	77.33 ^{ab}	4.600 ^{ab}	296 ^{ab}	76 ^{a}	7.167 ^{a}	8.500 ^{b}	98.33 ^{b}
80 mm × three times foliar application	100.00 ^{a}	2.267 ^{a}	81.33 ^{a}	4.667 ^{a}	310 ^{a}	90 ^{a}	7.500 ^{a}	10.670 ^{a}	100.00 ^{a}
160 mm × control	78.33 ^{c}	1.520 ^{e}	62.67 ^{c}	3.480 ^{c}	236 ^{b}	30 ^{a}	4.167 ^{d}	6.333 ^{c}	94.33 ^{d}
160 mm × two times foliar application	83.33 ^{c}	1.767 ^{d}	62.67 ^{c}	3.567 ^{c}	243 ^{b}	36 ^{a}	4.167 ^{d}	7.333 ^{bc}	95.33 ^{d}
160 mm × three times foliar application	83.33 ^{c}	1.963 ^{c}	62.67 ^{c}	3.737 ^{c}	236 ^{b}	43 ^{a}	5.500 ^{c}	8.000 ^{b}	97.67 ^{c}

Means of each group with the same letters are not significantly different compared to Duncan method.

In laboratory germination test, germination percentage, rate of germination and seedling dry weight were affected by irrigation regimes at 5% probability level and seedling fresh weight, length of root and seed vigour were affected at 1% probability level, although soil moisture status had no significant effect on length of root (Table 3). The maximum germination percentage (76.88%) and rate of germination (4.490), the highest seedling fresh weight (299 mg), the highest seedling dry weight (79 mg), length of shoot (7 cm) and most seed vigour (98.66%) were observed to irrigation after 80mm evaporation treatment, despite the lowest amount of above traits were belonged to irrigation after 160mm evaporation treatment (Table 4). On the other hand, foliar application of micronutrients didn't affect germination percentage, rate of germination and seedling fresh weight (Table 3). But, affected seedling dry weight, length of shoot and root and seed vigour at 1% probability level. The highest seedling dry weight (66 mg), length of shoot (6 cm), length of root (9 cm) and seed vigour (98.33%) were related to three times foliar application of micronutrients over the control (Table 4). Irrigation after 80 mm evaporation compared with irrigation after 160 mm evaporation increased seed vigour by 3 percent. On the other hand, foliar application of micronutrients has markedly improved seed vigour. Three times foliar application of micronutrients by comparison with control also significantly increased seed vigour (by 2%). It may be due to their act as a carrier of needed nutrients particularly into the seed as well as activator of enzymes like transphosphorylase, dehydrogenase and carboxylase as magnesium involved in carbohydrate metabolism, synthesis of nucleic acid (Hazra and Som, 1999). Similar increase in seed quality parameter were also reported by Rathinavel *et al*. (2000) and Sasthri *et al*. (2000) in cotton, Rubes (1984) in pea Moreover, interaction of irrigation regimes and foliar application of micronutrients had no significant effect on all above traits. Influence of foliar application of micronutrients is considerably due to the impact of them on rising rate of photosynthesis, increasing assimilates translocation from sources to sinks, increasing plant resistance to pests and diseases, improve water use efficiency and lastly increasing boll number per plant that cause in increasing cotton yield and seed's biological parameters. These results were in good accordance with those obtained by Christos Dordas (2006).

4. Acknowledgement

The valuable cooperation, land and needed resources provided by Islamic Azad University of Khorasgan Branch, by which I would like to thank university chairman and other professors, those who have supported me for doing this research.

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