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Effect of Pre-Sowing Humidification Treatment on Cottonseed Vigour

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Abstract

Seed of an upland cotton (*Gossypium hirsutum* L.) cv. NIAB-78 were incubated for 24, 48 and 72 h at saturated relative humidity. The vigour of treated seeds was studied under nethouse experimental conditions. Humidification did not affect emergence, seedling weight, and seedling length and time to 50% germination (T_{50}). Mean germination time was significantly reduced in seeds subjected to 72 h humidity. Electrical conductivity (EC) of seed leachates of all the treated seeds was lesser than that of control upto 4 h of soaking. However, EC of all treated seeds after 24 h was higher than that of control. There was no significant change in the acids values and free fat acidity in treated and untreated seeds but peroxide value of treated seeds was significantly lower than untreated seeds. It is concluded that cottonseed invigoration could not be improved under present experimental conditions and the genetic material.

Key Words: Cotton. Humidification, Seed invigoration

Introduction

Cotton is the world most important cash crop, grown in more than eighty countries of the world for its fibre. Low seed vigour is typical for many agronomic crops that result in low production. Slow, asynchronous, and unreliable germination and emergence arise due to low vigour seeds, which lead to problems for successful crop growth. If seeds germinate erratically over a long period of time, then the growth of the seedlings will not be uniform and the plants will mature over a wide period of time. The effects of seed vigour on emergence and stand establishment are well documented (Heydecker, 1977). Germination and vigour are at the highest when seed is at its maximum dry weight, a stage known as physiological maturity in most crops (TeKrony and Egli, 1997), however, like any other form of life, they cannot retain this identity indefinitely.

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Seed is seldom planted immediately after harvesting; it is stored for a few days, weeks, months or years. After harvest, seeds start deteriorating, moving inexorably towards death (Gregg *et al.*, 1994). During deterioration, vigour is the first component of seed quality, which is lost, followed by a loss of germination capacity and viability (Trawatha *et al*., 1995).

Seed invigoration treatments have, therefore, been developed to improve seed performance during germination and emergence. Most of these involve a period of controlled hydration of the seed to a point close to, but before, the emergence of the radicle after which the seeds are dried back to their initial moisture content before sowing (Khan, 1992; Basu, 1994). These invigoration treatments can enhance the transition from seed to seedling and improve germination rate and seedling vigour. The purpose of these treatments is to shorten the time between planting and emergence, and to protect seeds from biotic and abiotic factors during critical phase of seedling establishment. Such treatments synchronize emergence, which leads to uniform stand and improved yield.

Such treatments include osmoconditioning with polyethylene glycol (PEG) or a salt solution (Heydecker *et al*., 1975; Heydecker and Coolbear, 1977; Knypl and Khan, 1981), matriconditioning with solid matrix carriers (Taylor *et al.*, 1988; Gray *et al*., 1990; Hardegree and Emmerich, 1992; Beckman *et al*., 1993), humidification where seeds are hydrated at high relative humidity (Perl and Feder, 1981; Finnerty *et al*., 1992; Van Pijlen *et al.*, 1996; Lee *et al.*, 1998; Basra *et al.*, 2002), aerated hydration in which seeds are imbibed in aerated water for specific period and hydropriming where seeds are soaked in water and dried before sowing (Coolbear and McGill, 1990; Powell *et al.*, 2000; Soon *et al*., 2000). Expect hydropriming these treatments control the imbibition rate which has benefited many agronomic species and is being used for crops where improved seedling vigour and synchronous seedling emergence are desired (Bradford, 1986; Khan, 1992).

The main goal of these hydration techniques is to slow down the imbibition rate. This controlled hydration helps in better genetic and structural repair and trigger to the pre-germination metabolic activities. Such seeds when planted results in early and synchronized germination under a wide range of field conditions. Pre-

sowing humidification can be an alternate to the more popular techniques (Lee *et al.*, 1998). Humidification is the control of seed hydration by exposure of seeds to water vapor in indirect contact with water (Khan, 1992). Exposure of seeds to high relative humidity leads to increase in seed moisture content below the threshold required for germination (Johnson-Flanagen *et al*., 1994). As a result, the water uptake is restricted by humidification as by osmoconditioning during early phases of imbibition (Johnson-Flanagen *et al*., 1994). It has been reported that humidification reduced the imbibitional chilling injury in cotton (Thomas and Christiansen, 1971), lettuce and sunflower (Ellis *et al*., 1995). However, this treatment has the danger of seed deterioration during the prolonged period of humidification (Hegarty, 1978).

Practically, humidification is applied in seeds of tomato (Pen aloza and Eira, 1993), jute (Chowdhury and Choudhuri, 1987), eggplant and radish (Rudrapal and Nakamura, 1988), peas (Siviritepe and Dourado, 1994), and rice (Lee *et al.*, 1998), but information is limited for cottonseed (Thomas and Christiansen, 1971). Therefore, humidification effect on cottonseed was studied to improve emergence.

Methodology

Cultivar NIAB-78 of upland cotton (*Gossypium hirsutum* L.) was used in the study. The seed was obtained from Nuclear Institute for Agriculture and Biology (NIAB), Faisalabad. The experiments were conducted in the laboratories of the departments of Crop Physiology and Biochemistry, University of Agriculture, Faisalabad.

Humidification treatments

Delinted cottonseeds were exposed to saturated humidity for controlled hydration following the method described by Johnson-Flanagan (1994) with minor modifications. For each treatment 250 g of seeds were placed on plastic trays in a Plant growth cabinet (Type 8194, VINDON, England). The relative humidity and temperature in the cabinet were maintained between 95-100% and 24-26ºC, respectively. The seeds were exposed to these conditions for 24, 48 and 72 h.

Post humidification operations

With humidification treatments, weight of the seeds increased (9-15% dry weight basis). The seeds were dried on the laboratory benches under shade by forced air for 2 days, and stored in sealed polythene bags at 6-8ºC until required (Alvarado *et al*., 1987).

Seed moisture adjustment

The treated and untreated seeds were placed in a sealed drying cabinet (LEEC Drying Cabinet, Model F2, LEEC Ltd., England) at 25ºC for 3 days. The seeds attained moisture of 8%. The seeds were sealed in polythene bags and placed in refrigerator at 8±2ºC for later studies.

Seed vigour evaluation

Equal amounts of garden loam soil and compost were mixed thoroughly and sieved to remove any large particles. For this experiment, 15 cm diameter and 30 cm deep garden (earthen) pots were used. The pots were placed in a nethouse under natural sunlight. Each pot was flooded with equal amount of water. After four days when the soil reached field capacity, 5 seeds per pot were sown in 2.5 cm deep holes. The experiment was replicated thrice. Water was applied when the consistency of the soil was such that the ball formed by squeezing it in the palm of the hand was easily broken when pressed between two fingers (ISTA, 1985). A seed was considered as emerged when cotyledons were visible. After 22 days of sowing, the pots were watered and seedlings were carefully uprooted to record the following data: (i) Final emergence percentage; (ii) T_{50} - days to 50% emergence; (iii) Mean emergence time (MET) {Ellis and Roberts (1981)} using the following equation:

$$MGT = \frac{\sum Dn}{\sum n}$$

where *n* is the number of seeds which emerged on day *D* and *D* is the number of days counted from the beginning of the test}; (iv) Cotyledon area; (v) Cotyledon fresh weight; (vi) Cotyledon dry weight; (vii) Shoot length; (viii) Shoot fresh weight; (ix) Shoot dry weight; (x) Hypocotyl length.

Dry weights of shoots or cotyledons were obtained by overnight drying in an oven at 85ºC (ISTA, 1985).

Electrical conductivity (EC) of seed leachates

Solute leakage of the seeds was estimated by soaking 1 g seed of each treatment in 50 mL of deionised water at 25ºC in an incubator (LMS Cooled Incubator, LMS Ltd., Kent, England). Before soaking, the seeds were rinsed in deionised water to remove any salt or dust deposition and then dried by filter paper. The experiment was replicated for 3 times. The electrical conductivity of seed leachates was measured by conductivity meter (Twin Cond. Conductivity Meter, B-173, Horiba Ltd., Miyanohigashi, Kisshoin, Kyoto, Japan) after 0.25, 0.5, 2, 4, and 24 h of soaking (Bailly *et al.*, 1996; Sung, 1996). The conductivity of soaked solution was expressed per gram of seeds (mS cm^{-1} g^{-1}).

Biochemical analysis

Oil was extracted by solvent extraction method using diethyl ether as solvent according to AOCS, Aa 4-38 (1990).

Analysis of seed oil

Oil obtained was filtered and used for the determination of the following parameters employing the official methods of AOCS (1990): (i) Acid value, AOCS, Cd

3a-63 (1990); (ii) Percentage of free fatty acids, Aa 6-38 AOCS (1990); (III) Peroxide value, AOCS Cd 8-53 (1990).

Statistical analysis

Emergence test was arranged in a completely randomized design. The data were analysed using a statistical package, MSTAT-C. The recorded data were analysed statistically using Fisher's analysis of variance techniques and LSD test was applied at 5% probability level to compare the differences among treatment means (Steel and Torrie, 1984). The oil analysis results were expressed as the means and standard errors were calculated from the replicates.

Results

Pre-sowing seed humidification did not effect significantly final emergence percentage, however, MET was effected significantly. There was no effect of single day humidification on MET. Humidification for two days significantly increased the mean germination time while increase in further humidification period significantly decreased it (Table 1). Incubation of cottonseeds to saturated humidity did not affect the time taken to 50% emergence.

Pre-sowing seed humidification treatments had insignificant effect on shoot length, however, decreased significantly shoot fresh and dry weights. The shoot fresh weight of all the humidification treated seeds were statistically similar. A similar trend was observed in case of shoot dry weight (Table 1). Pre-sowing seed humidification had no effect on cotyledon area and cotyledon fresh or dry weights (Table 1). Pre-sowing seed humidification had insignificant effect on hypocotyl length.

Electrical conductivity of seed leachates

Electrical conductivity of all the seed increased with soaking period. All the humidification treatments had slightly higher EC values after 15 minutes (0.25 h) of soaking period. However, with increase in soaking period up to half an hour resulted in less solute leakage than the control. A similar trend of low EC than that of control was observed up to 4 h of soaking. While after 24 h of soaking time, the EC of solute leakage of all the humidification treated seeds were slightly higher than that of control (Fig. 1).

Oil analysis of cottonseeds

Different humidification treated seed oils had different acid values (Table 2). Acid value of seed oil increased by one day of humidification treatment and then linearly decreased beyond this treatment period. Minimum acid value (1.12) was recorded from three days humidified seeds. Pre-sowing seed humidification up to two days increased free fatty acid contents. Seed hydration by humidification beyond this period resulted in reduction in free fatty acid contents of seed oil (Table 2). Increase in pre-sowing exposure of seeds to saturated humidity decreased the peroxide value. Minimum peroxide value (2.0) was recorded from oil of three days treated seeds (Table 2).

Discussion

Controlled hydration offers an effective mean for raising seed performance in many crop species. Exposure of seeds to high relative humidity leads to controlled increase in seed moisture as by osmoconditioning (Perl and Feder, 1981; Finnerty *et al*., 1992; Johnson-Flanagen *et al*., 1994; Suzuki and Khan, 2001). Humidification is also used for controlled hydration before sowing to avoid imbibitional injury under low temperature sowing (Thomas and Cristiansen, 1971; Ellis *et al*., 1995).

Pre-sowing seed hydration by humidification did not effect emergence percentage under present experimental conditions. These results are in line with earlier research. No abnormal seedling was recorded. Humidification significantly decreased seedling fresh and dry weight, while shoot length remained unaffected. The present data confirm the earlier research (Bai *et al*., 1997), which indicated that even though the seed moisture of Wyoming big sagebrush content reached as high as 60% after humidification, total germination percentage, time to 50% germination and, seedling vigour did not change. He concluded that germination is related more to habitat or genetic variations than the initial moisture contents and manipulating seed moisture might not be beneficial. Suzuki and Khan (2000) reported that seed vigour was improved by humidification as measured by enhanced germination, ACC (1-aminocyclopropane-1-carboxylic acid)-derived ethylene production, and seedling emergence and growth in snap bean, which are not supported by the present results. Hobbs and Obendorf (1972) also reported that germination in soybean was enhanced by seed moisture manipulation.

Electrical conductivity showed inconsistence results. There was linear increase in the EC of all seed leachates. All treated seeds showed higher EC value than control after 24 h of soaking. EC test measures the electrical conductivity of water in which seeds have been soaked. Conductivity measurement is related to germination, emergence potential and early seedling growth.

Thomas and Christiansen (1971) reported that in cottonseed, there was no improvement in emergence over the control by preconditioning of good quality seeds. They indicated that high quality cottonseeds did not respond to preconditioning, as did the low vigour seeds. It might be a reason that humidification was ineffective because of high vigour seeds used in the present study (83.33% emergence of controlled seeds).

Table 1: Influence of pre-sowing humidification treatment on emergence and seedling vigour of cottonseed cv. NIAB-78 sown under nethouse conditions.

Humidification period (h)	Emergence (%)	MET (days)	T_{50} (days)	Shoot fresh weight (g)	Shoot dry weight (g)	Shoot length (cm)	Cotyledon area (cm^2)	Cotyledon fresh weight (g)	Cotyledon dry weight (g)	Hypocotyl length (cm)
Control	83.33	3.59b	3	1.573a	0.12a	14.18	10.02	0.54	0.03	11.60
24	73.33	3.523b	3	1.15b	0.09b	14.77	10.06	0.48	0.02	12.30
48	73.33	4.04a	3	1.133b	0.08b	14.97	10.47	0.45	0.03	12.86
72	86.67	3.08c	3	1.017b	0.08b	14.77	9.57	0.48	0.02	12.96
LSD	14.38	0.399	NS	0.238	0.0066	NS	NS	NS	NS	NS

The letters with different alphabets are statistically different at P = 0.05; MET = emergence time; NS = non significant

Table 2: Oil analysis of cottonseed cv. NIAB-78 as influenced by pre-sowing humidification

Humidification period (h)	Acid value	Free Fatty Acid (%)	Peroxide value
Control	2.81 ± 0.005	1.4 ± 0.003	6.36 ± 0.014
24	3.36 ± 0.017	1.7 ± 0.009	2.89 ± 0.382
48	2.24 ± 0.023	1.1 ± 0.011	2.49 ± 0.372
72	1.12 ± 0.011	0.55 ± 0.006	2.06 ± 0.285

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