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Cottonseed Invigoration by Pre-Sowing Seed Humidification

SHAHZAD MAQSOOD AHMAD BASRA, NAZIR AHMAD, KHALIL-UR-REHMAN† AND NADIA IQBAL
Departments of Crop Physiology and †Biochemistry, University of Agriculture, Faisalabad-38040, Pakistan

ABSTRACT

Vigour changes by pre-sowing humidification of an upland cotton (*Gossypium hirsutum* L.) cv. NIAB-78 were studied under laboratory conditions. The seeds were exposed to saturated relative humidity at 25ºC for 24, 48 and 72 h. The treated seeds were compared with untreated seeds for vigour evaluation using the standard procedures under laboratory conditions. The germination percentage decreased with increase in humidification period. Humidification significantly increased the seedling length, while seedling fresh and dry weights remained unaffected. Electrical conductivity (EC) of seed leachates decreased with humidification than that of control up to 4 hours of soaking. However, after 24 hours of soaking treated seeds had higher EC values than that of control. These results reveal that cottonseed invigoration could not be achieved by seed humidification under the present experiment material and conditions.

Key Words: Cottonseed; Priming; Humidification; Invigoration

INTRODUCTION

Rapid and uniform germination is as important for better crop production, as is the total germination. 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. Germination and vigour are highest when seed is at its maximum dry weight, a stage known as physiological maturity in most crops (TeKrony & Egli, 1997), however, like any other form of life, they cannot retain this identity indefinitely. 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).

Slow, asynchronous, and unreliable germination and emergence arise due to low vigour seeds, which lead to problems for successful crop growth. 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). 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 which ensures synchronize emergence, uniform stand and improved yield.

Such treatments include osmoconditioning (Heydecker & Coolbear, 1977; Knypl & Khan, 1981), matriconditioning (Taylor *et al.,* 1988; Hardegree & Emmerich, 1992), humidification (Finnerty *et al*., 1992; Van Pijlen *et al.,* 1996; Lee *et al.,* 1998), aerated hydration and hydropriming (Powell *et al.,* 2000; Soon *et al*., 2000). Humidification is a pre-sowing hydration treatment in which seeds are equilibrated under conditions of high humidity (Finnerty *et al*., 1992; Van Pijlen *et al.,* 1996; Suzuki & Khan, 2001). Humidification leads to controlled increased in seed moisture as by osmoconditioning (Finnerty *et al*., 1992; Johnson-Flanagen *et al*., 1994; Suzuki & Khan, 2001). It is also used for controlled hydration before sowing to avoid imbibitional injury under low temperature sowing (Thomas & Cristiansen, 1971; Ellis *et al*., 1995).

Seed preconditioning has been extensively used in temperate horticultural and agronomic crops. However, there is dearth of information about preconditioning of oilseeds especially that of cotton. So it was imperative to develop suitable techniques for improving cottonseed germination capacity. The present study was carried to test pre-sowing humidification in cottonseed as an invigoration technique.

MATERIALS AND METHODS

The experiment was conducted in the laboratory of the Department of Crop Physiology, University of Agriculture, Faisalabad, Pakistan, during 1999-2000. Cultivar NIAB-78 of upland cotton (*Gossypium hirsutum* L.) was used in the study. The seed was obtained from the Nuclear Institute of Agriculture and Biology (NIAB), Faisalabad.

The lint on the seeds was removed by thorough mixing of seeds with commercial grade H_2SO_4 in plastic containers @ 1 L H_2SO_4/10 kg cottonseed. Immediately after delinting, the seeds were thoroughly rinsed with deionised water and dried under shade by forced air for 2 h.

Humidification treatment. The seeds were surface sterilized before seed treatment. The seeds were treated with 5% (v/v) sodium hypochlorite solution for 3 minutes to

avoid fungal invasion (Rajanna & de la Cruz, 1977) followed by thorough washing with deionised water and drying using tissue paper. 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 the following periods of time: 0 (control), 24 (Hd_{24}), 48 (Hd_{48}) and 72 (Hd_{72}) hours.

Post humidification operations. After humidification the seeds were dried on the laboratory benches under shade by forced air for 2 days (Alvarado *et al*., 1987). The treated and untreated seeds were placed in a sealed drying cabinet at 25ºC for 3 days and then sealed in polythene bags and placed in refrigerator at 8±2ºC for later studies.

Seed vigour evaluation. To record the changes in seed vigour caused by the seed treatments, the treated seeds were compared with control for following various vigour tests:

Standard germination test. The standard germination test was performed in a germinator with minor modifications according to AOSA (1991). The seeds were surface sterilized with 1% sodium hypochlorite solution for 5 seconds to avoid fungal attack and thereafter, rinsed 4 times with deionised water (Toorop *et al*., 1998). Filter paper sheets of 24" x 24" were used for germination test. Twenty seeds were placed on these marks with radicle side downward, covered with another sheet same size and moistened with deionised water. The sheets were rolled and placed upright in a less-than-airtight container to prevent papers from drying too rapidly so as to maintain high humidity, and to provide proper aeration to germinating seeds. The containers were incubated with alternating temperatures between 20°C for 16 h and 30°C for 8 h. Each treatment was replicated three times. To record data, the sheets were unrolled 4, 8 and 12 days after planting, and the seeds that had produced normal seedlings were counted and recorded. At this time, one count of normal seedlings that had a combined hypocotyl and root length of 1.5 in or longer was made (AOSA, 1991). Following observations were recorded after 12 days of planting: (i) Final germination percentage (%); (ii) seedling length - the root-hypocotyl measurement was made from the point of cotyledon attachment to the tip of the radicle (cm); (iii) Seedling fresh weight (g) and (iv) seedling dry weight (g).

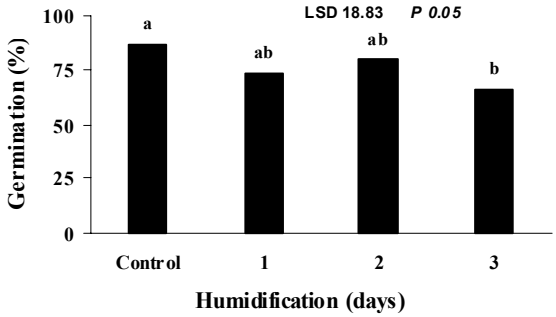
Electrical conductivity of seed leachates. Solute leakage of the seeds was estimated by soaking 1 g seeds in 50 mL of deionised water at 25ºC in an incubator (LMS Cooled Incubator, LMS Ltd., Sr. No. 1391/89L, The Modern Forge, Riverhead, Sevenoaks, Kent, England). Before soaking, the seeds were rinsed 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 15, 30 and 60 min and then after 2, 4, 6 and 24 h of soaking (Bailly *et al.,* 1996). The conductivity of soaked solution was expressed per gram of seeds (mS/cm/g).

The experiment was arranged in a completely randomized design. The data were analyzed using a statistical package, MSTATC. The recorded data were analyzed 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 & Torrie, 1984).

RESULTS

Germination. Pre-sowing humidification had significant effect on cottonseed germination (Fig. 1). The control and humidified up to two days seeds had significantly similar and maximum germination percentage. Minimum germination (66.67 %) was recorded from seeds subjected to pre-sowing humidification for three days. No abnormal seedlings were found.

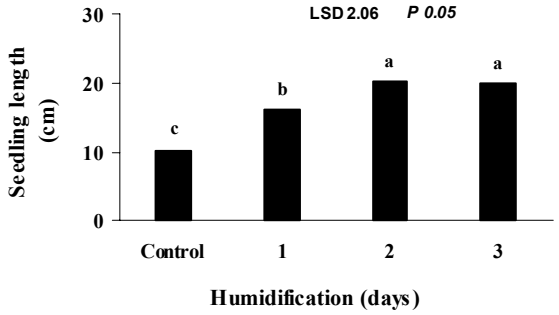
Fig. 1. Influence of presowing humidification on germination of cotton cv. NIAB-78. The bars with different alphabets are statistically different



Seedling length. Pre-sowing humidification significantly affected the seedling length (Fig. 2). A significantly increasing trend in seedling length was observed by seed humidification. The seedling lengths of seed humidification treatments for two and three days were statistically maximum and at par with each other. Minimum seedling length (10.33 cm) was recorded from the control.

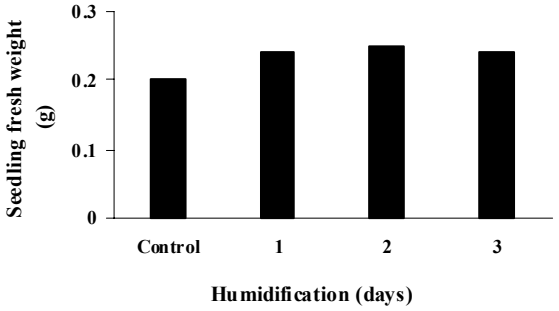
Seedling fresh and dry weight. Pre-sowing seed humidification had no effect on seedling fresh and dry weights (Fig. 3, 4).

Fig. 2. Influence of presowing humidification on seedling length cotton cv. NIAB-78. The bars with different alphabets are statistically different



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 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 four hours of soaking. While after 24 hours of soaking time the EC of solute leakage of all the humidification treated seeds were slightly higher than that of control (Fig. 5).

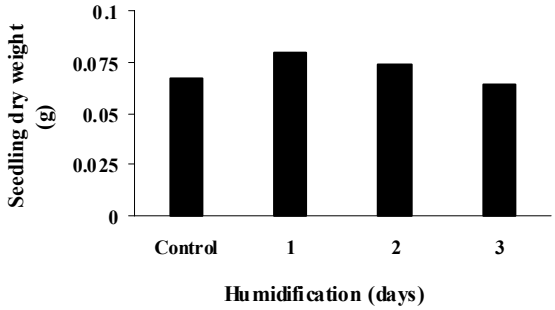
Fig. 3. Influence of pre-sowing humidification on seedling fresh weight of cotton cv. NIAB-78



DISCUSSION

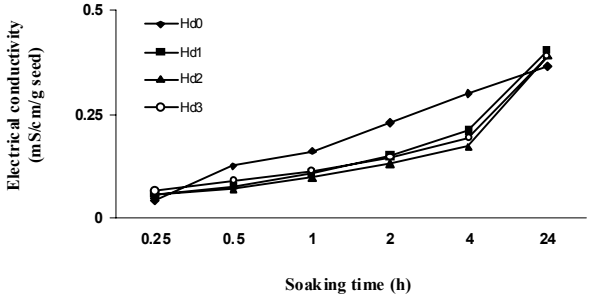
Exposure of seeds to high relative humidity leads to increase in seed moisture content below the threshold required for germination (Johnson-Flanagen *et al.,* 1994). Water uptake in seeds occurs in three distinct phases: (i) imbibition, (ii) a lag phase, and (iii) visible germination (Copeland & McDonald, 1995). Phase I can occur in dead seeds and is independent of metabolic activity, while phase II is a period of active metabolism leading to radicle emergence and subsequent substrate mobilization during phase III. As a result the water uptake is restricted by

Fig. 4. Influence of pre-sowing humidification on seedling dry weight of cotton cv. NIAB-78



humidification as by osmoconditioning during early phases of imbibition (Johnson-Flanagen *et al.,* 1994). Metabolic activity increases in seeds with hydration. This results in increase in respiration rate, enzyme activation (Schopfer & Plachy, 1984), builds up of ATP and increase in protein synthesis (Johnson-Flanagen *et al.,* 1994). Controlled

Fig. 5. Influence of pre-sowing humidification on electrical conductivity of cottonseed cv. NIAB-78



imbibition has been used to improve seedling vigour and synchronize seedling emergence in a variety of species (Hydecker & Coolbear, 1977; Khan 1992). It has been reported that humidification reduced the imbibitional chilling injury in cotton (Thomas & Christiansen, 1971), lettuce and sunflower (Ellis *et al.,* 1995).

There was a decreasing trend in final germination count with increase in humidification time (Fig. 1). These results are in line with earlier research (Bai *et al.,* 1995; Bai *et al.,* 1997). No abnormal seedling was recorded. Humidification significantly increased the seedling length (Fig. 2), while seedling fresh and dry weights remained unaffected (Fig. 3,4). Bai *et al.* (1995) found that humidified seeds did not show a consistent difference from non-humidified seeds for germination rate, or seedling vigour. 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 changed. He concluded that germination is related to

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 results. Hobbs and Obendorf (1972) also reported that germination in soybean was enhanced by seed moisture manipulation.

The failure of invigoration of cottonseed by humidification in the present study confirms the findings of Thomas and Christiansen (1971) who reported that in cottonseed, 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 a reason that humidification was ineffective because of high vigour seeds used in the present study.

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