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Short Communication

Methanol Stress Test to Evaluate Initial Seed Vigor in Cotton

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In spite of high germination percentage, some seed lots show poor field performance expressed as low seedling vigor and susceptibility to diseases and environmental stresses resulting in a poor plant stand. Accelerated Ageing method devised for predicting storabilility of seeds is a widely used technique in seed vigor testing [1]. In cotton, the method involves exposure of seeds to 42 ± 0.5°C temperature and 100 per cent relative humidity for 72 hours [2, 3]. Major disadvantage of this technology is the length of time required and its associated microbial contamination leading to errors in findings. There are other stress tests utilizing hot water or methanol solutions which have been found to mimic the effect of Accelerated Ageing and may be useful as a screening tool for evaluating seed quality. Hot water seed treatment has been proposed for cotton seed deterioration [3] which was later confirmed suggesting hot water treatment to be more effective, rapid and uniform in deteriorating cotton seed [4, 5]. Methanol stress test have also been studied extensively and proved useful in seed vigor evaluation of soybean [6, 7] and Kenaf [8]. In cotton, one study suggests 20 per cent methanol treatment for six hours as a useful seed vigour test [9].

Cotton genotypes of *G. hirsutum* including varieties and hybrids released in central zone such as LRK 516, CNH 36, CNH 120MB, Hybrid 8, NHH 44 and its parents, AC-738 and BN-1 and *G. arboreum* such as AKA-7 and AKA-8401 as well as few promising *arboreum* cultures viz. CINA 345, CINA 346, CINA 347, CINA 348 and CINA 349 were studied for their response towards methanol stress, to differentiate them for initial seed vigour. Fresh seeds of above genotypes were obtained which were acid delinted using 1 per cent sulphuric acid. They were tested for their initial moisture content and dried to bring down the moisture content to near uniformity (6-8%). Their initial germination were above 60 per cent. The genotypes could not be definitely categorized for their response to 10 per cent or 20 per cent methanol treatment given for 2 hours/4 hours/6 hours (data not given) as reported earlier. Hence, the seeds were soaked in 30 per cent methanol solution for a period of two hours. Washed seeds were later subjected to germination assay [10] on rolled paper towels keeping 100 seeds in four replications along with control. The test revealed considerable variation for response among the genotypes. Genotypes of *arboreum* including promising cultures and released varieties exhibited low or non-sensitivity towards methanol stress (Fig. 1) and were classified as high vigour seeds. Among *hirsutums*, germination of CNH 36, NHH 44 and its parents viz. AC 738 and B.N-1 were highly affected due to methanol stress classifying them to be in low seed vigour category. Since vigour is a complex trait, its assessment by a single test may not be complete and hence above classification was further confirmed by other tests too. It is understood that weakening of membrane integrity is a component of stress response/low vigour resulting in increased leakage of cellular constituents and increased peroxidation of unsaturated lipid fraction in cell membrane. The seeds were therefore subjected for estimation of electrical conductivity (E.C.) of seed leachate as well as level of lipid

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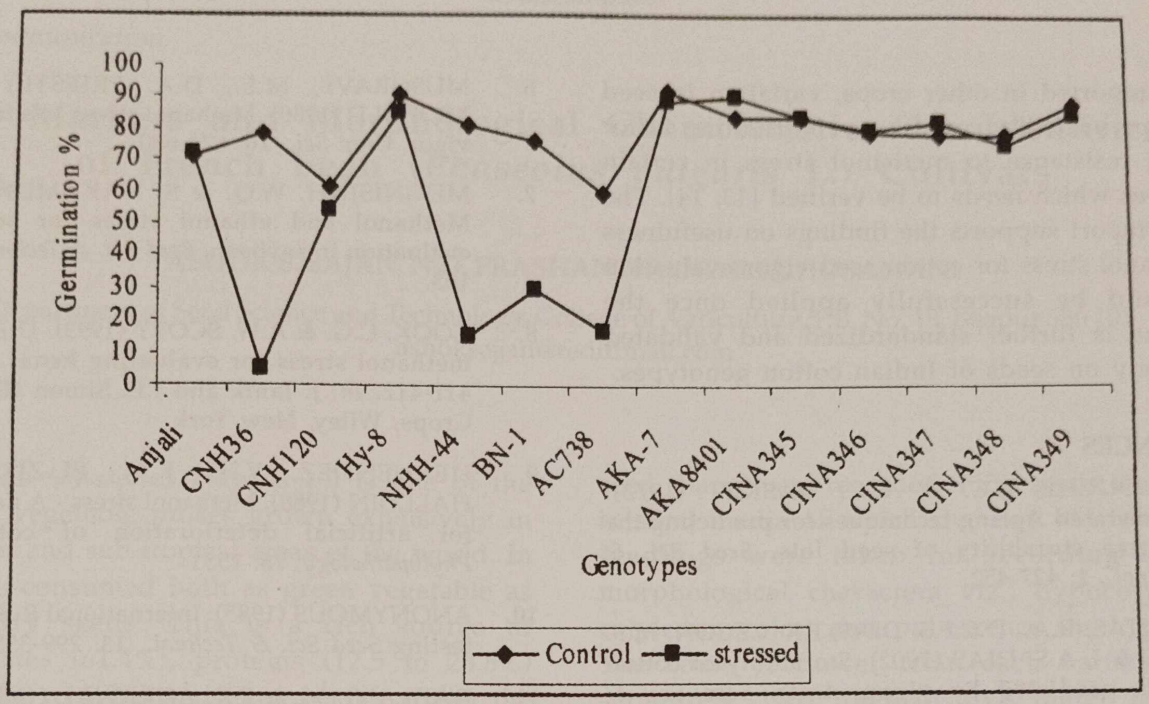

Fig. 1. Germination response of cotton genotypes towards methanol stress

peroxidation measured by amount of malonaldehyde released. Four replications of 25 seeds each were soaked in a glass beaker containing 15 ml distilled water and kept at 25°C in an incubator for 17h. E.C. of the solution was then measured by a conductivity meter [11]. Quantity of malonaldehyde released during lipid peroxidation was determined using TBA-TCA reagent and measured spectrophotometrically [12]. The results of both the tests were almost in line with those of methanol stress test. The genotypes earlier classified in low vigor category consistently showed high values for both electrical conductivity and lipid peroxidation (Fig. 2).

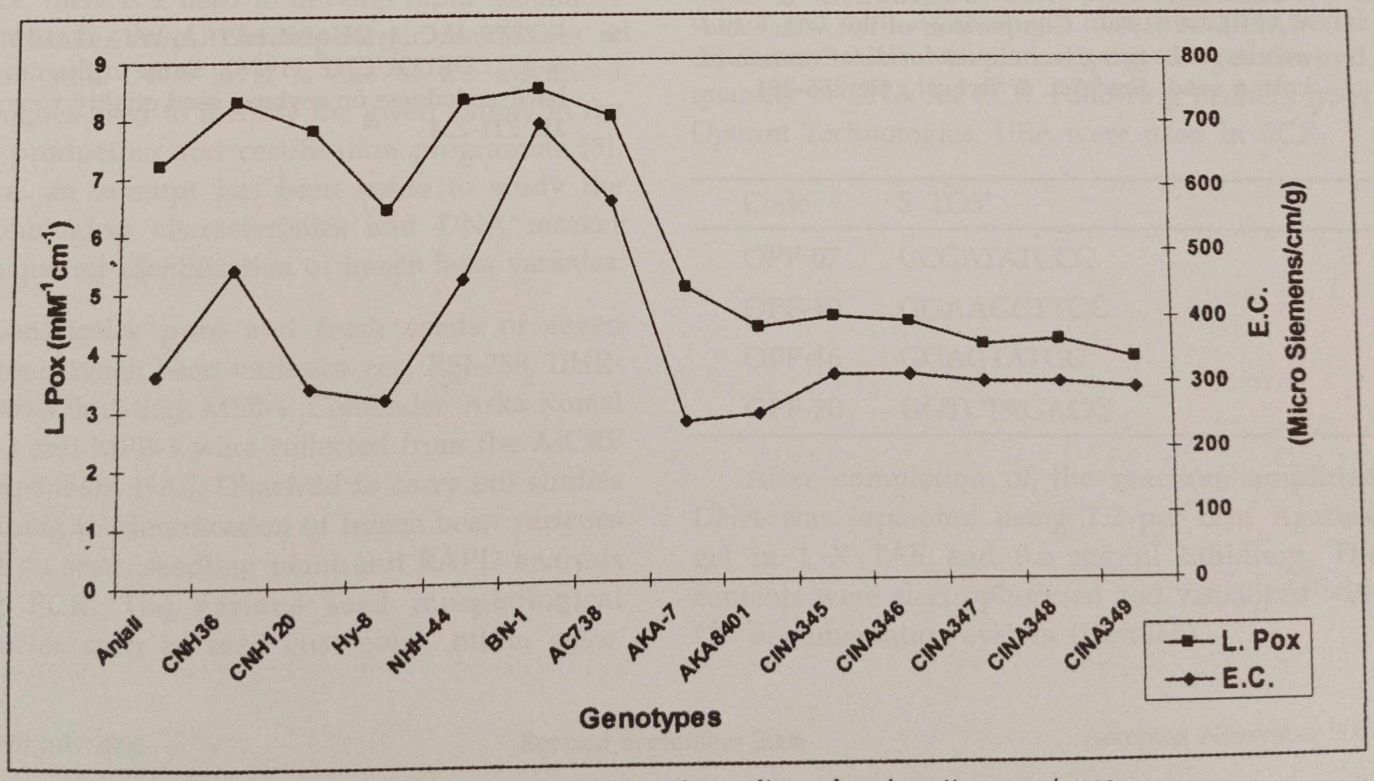

Fig. 2. Electrical conductivity and lipid perodixe values in cotton genotypes

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As reported in other crops, variation in seed coat impermeability could be a contributing factor offering resistance to methanol stress in certain genotypes which needs to be verified [13, 14]. The present report supports the findings on usefulness of methanol stress for cotton seed vigor evaluation and could be successfully applied once the technique is further standardized and validated extensively on seeds of Indian cotton genotypes.

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