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IDENTIFICATION OF COTTON GENOTYPES THROUGH CHEMICAL TESTS

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Research Article

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ABSTRACT

An experiment was carried out to characterize and to identify three cotton hybrids and its parents viz., HBB-224 (LRA 5166 x P 4, Surya (T 13 x M 12 and Savitha (T 7Xm 12) and four varieties viz., Surabhi, Anjali, Supriya and SVPR-2 based on the chemical tests (standard phenol test, modified phenol test, GA_3 test, KOH test, NaOH test, 2,4-D test and hydrogen peroxide test). Among the various chemical tests, sodium hydroxide test, potassium hydroxide test and hydrogen peroxide test gave the stable results and can be effectively used for cultivar differentiation. The study revealed that these tests could be effectively used for determining the varietal purity of cotton genotypes for routine testing in seed testing laboratories as some of the cultivars showed distinct response to these chemical tests.

KEYWORDS: *cotton, chemical test, KOH test, NaOH test*

INTRODUCTION

Cotton (*Gossypium spp*.) is the most important textile fiber crop in the world and it is considered as "king of fiber crop". It plays an important role in national and international economy. India is the second largest cotton producer in the world next to China with cultivable area of 111.42 lakh ha with the production of 329 lakh bales of 170 kg and the productivity of 518 kg/ha. Cotton belongs to the family Malvaceae and the genus *Gossypium* with about 49 species (Percival and Kohel, 1990). Of the 49 species, only four species are cultivated in India. Crop improvement programmes in India have developed a large number of varieties in the last 30 years and originated from interspecific crosses of *G. hirsutum* resulted in the development of varieties with narrow genetic base. Continuous release of varieties has warranted to develop suitable techniques for varietal identification at the laboratory level particularly when the seeds have been submitted for seed purity analysis. Maintenance of genetic purity of varieties is more important for preventing varietal deterioration during successive regeneration cycles and for ensuring varietal performance at an expected level. Characterization of genotypes assumes importance with the implementation of Protection of Plant Varieties and Farmer Rights Act (PPV& FR), 2001. Cultivar can be identified by various methods *viz.,* morphological method, chemical method, biochemical method and molecular methods. Morphological method utilizes various morphological markers *viz.,* leaf characters, flower characters and fruit characters. Though, it is simple and easy method, it is tedious and time consuming. Many of the morphological traits possess multigenic expression which is altered by environmental factors. These limitations are overcome by rapid and reliable methods of varietal identification are to use of chemical tests. The chemical tests reveal differences among the seeds and seedlings of different varieties. These tests require virtually no technical expertise and can be completed in a relatively short time. The results of these tests are usually distinct, easily interpreted and help in grouping of the genotypes. Therefore, an investigation was carried out to ascertain the response of cotton genotypes to various chemicals to explore the possibility of using these tests for grouping and identification of cotton cultivars.

MATERIALS AND METHODS

The experimental materials for the present investigation consisted of three cotton hybrids *viz.,* HB 224, Surya and Savitha and its parents HB-224 (LRA 5166 x P 4, Surya (T 13 x M 12) and Savitha (T7 x M12) and four varieties *viz.,* Surabhi, Anjali, Supriya and SVPR-2. Genetically pure seeds of all the genotypes except SVPR 2 were obtained from Central Institute for cotton Research (CICR), Coimbatore and SVPR 2 was obtained from Regional Research Station, Srivilliputhur, Tamilnadu. Three hybrids and its parents were obtained from CICR, Coimbatore, Tamil Nadu. The following chemical tests were performed by the methods as suggested by Ram *et al.,* 2001 for rapid identification of cotton genotypes.

Phenol test:

The standardized phenol test for varietal purity testing as suggested by Shaista halim *et al.,* (1984) was followed. Four replications of 25 seeds were presoaked in distilled water for 24 h at 25° C. Then they were transferred on to two layers of germination paper saturated with one per cent phenol solution. The petri dishes were covered and incubated at 25° C and the colour reactions were noted after 24 h. Based on the development of seed coat colour, the selected cultivars were classified into different categories.

Modified phenol test:

The modified phenol test for varietal purity testing as suggested by Banerjee and Chandra, (1977) was followed. This test was conducted similar to standard phenol test except that four replications of 25 seeds were soaked in 3 per cent hydrogrn peroxide solution for 24 h. Then they were soaked in 0.4 per cent $CuSO_4$ solution for addition of Cu ions and 0.6 per cent sodium carbonate solution for addition of Na ions for 6 h each. Colour reaction was noted after 48 h of incubation and the cultivars were classified based on colouration of seed coat into different categories.

Sodium hydroxide test:

Four replications of 25 seeds of each genotype were soaked in 5% NaOH solution and kept at room temperature for 6 h. then the solution was observed for various colour development (Jawaharlal, 1994).

KOH test:

Four replications of 100 seeds in each genotypes were soaked in five per cent KOH solution for three hours and thereafter change in colour of the solution was observed. (Vanangamudi *et al.*1988b). Based on the intensity of the colour reaction, the genotypes were classified.

GA_3 test

One hundred seeds (25X4) were presoaked in 100 ppm GA_3 for a period of 24 hours and germinated as per ISTA (1996). Observations were recorded on 7th day in terms of increase in shoot length over that of control.

2,4 D test

The germination paper was moistened with 0.5 ppm concentration solution of 2,4 D. Four replications of 25 seeds of each genotypes were placed in 2,4 D moistened paper and germinated in roll towel method. The whole set up was in a germinator at 25^{0}C. After 7 days the seedling growth was observed. (Buttery and Buzzell, 1968).

Ferrous sulphate test

Four replications of 25 seeds of each genotype were soaked in 50 ml of 1% $FeSO_4$ solution for 2 h at 25^{0}C. Based on the seed color, development varieties were grouped (Bora *et al*. 2008).

Hydrogen peroxide soak test

Four replications of 25 seeds in each genotype were soaked in 3 per cent hydrogen peroxide solution for 5 h. Then the seeds were germinated in roll towel kept in germinator at 25°C. After 7 days the seedling growth was observed. (Buttery and Buzzell, 1968)

RESULTS AND DISCUSSION

The results obtained from the present study and discussions have been summarized below.

Varietal identification by morphological characters is laborious, time consuming, tedious, cumbersome and costly. A number of chemical tests have been developed for varietal identification such as phenol test, ferrous sulphate test, potassium hydroxide test, sodium hydroxide and peroxidase test. These chemical tests are very quick, easy and reproducible (Ashwani Kumar *et al.*, 1995). These tests provide supportive evidence for morphological evaluation of seeds (Vanderburg and Vanzwol, 1991) and aid in preparation of varietal identification keys.

The phenol and modified phenol tests did not stain different cotton genotypes rendering all genotypes undistinguishable and grouping was not possible. The reason attributed for lack of phenol color reaction may be due to the absence of tyrosinase enzyme in seed coat or lack of highly specific and

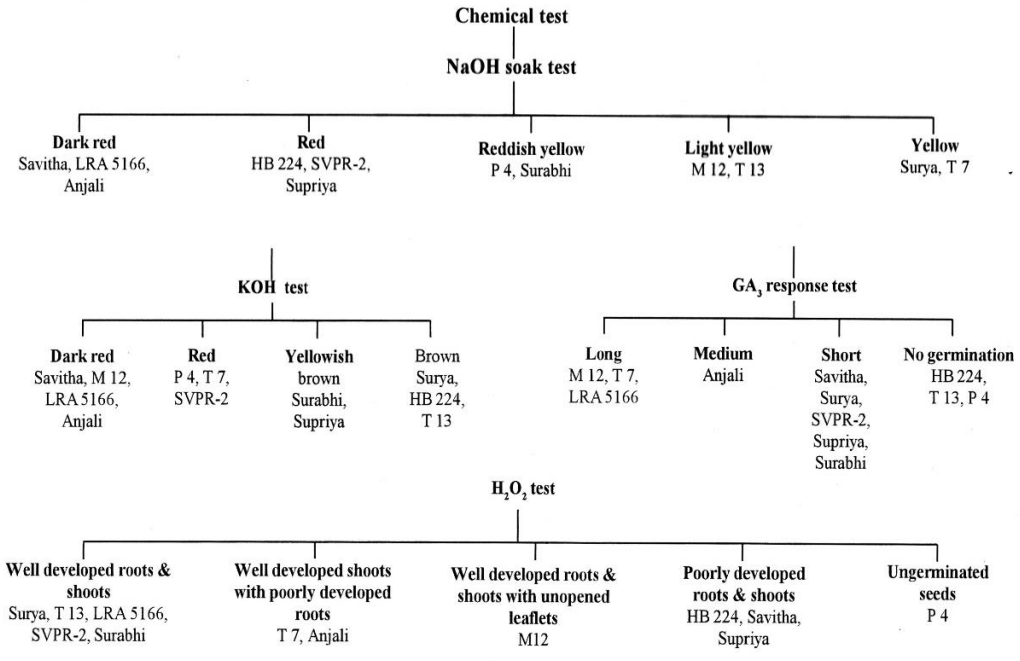
monogenically controlled response localized in seed coat. However, several researchers have successfully used phenol and modified phenol test of differentiating seeds of cotton varieties (Ponnuswamy *et al*., 2003), rice; Sivasubramanian and Ramakrishnan, 1974; Janaiah *et al*., 2003), in wheat (Singhal and Prakash, 1988; ISTA, 2004). Potassium hydroxide soaking led to varied color reactions of seed soak solution and the genotypes were grouped as dark red colour (Savitha, M12, LRA 5166 and Anjali), red colour (P 4, T 7 and SVPR 2), Yellowish brown colour (Surabhi and Supriya) and brown colour (Surya, HB 224 & T13) (Flow chart 1). This result is in accordance with the findings of Vanangamudi *et al.,(*1988). Varied colour reaction may be due to the chemical composition of seed or selective action of enzymes present which may be governed genetically.

Similarly, the sodium hydroxide soaking led to the genotypes being grouped as dark red colour (Savitha, LRA 5166, Anjali), red colour (HB 224, SVPR-2, Supriya), reddish yellow colour (P 4 and Surabhi), light yellow colour (M 12 & T 13) and yellow colour (Surya & T7) (Flow chart 1). Similar classification by NaOH test was reported earlier by Sambasivarao *et al*. (2002) in rice, Ponnuswamy *et al*. (2003) in cotton and Biradar Patil *et al.* (2006) in safflower genotypes. The colour reaction to sodium hydroxide solution was obtained due to reaction of seeds to secondary metabolites present in the seed coat and may be a stable genetic character (Vanderburg and Vanzwol, 1991).

The varied hypocotyl growth response of cotton genotypes to gibberllic acid (25 ppm) has been observed in the present study. Increase in hypocotyl length (total length between cotyledonary node and the base of the seedling) due to GA_3 varied significantly with the genotypes and the cotton genotypes studied were grouped as long (M 12, LRA 5166 &T 7), medium (Anjali), short (Surya, Savitha, SVPR -2 & Suprita & Surabhi) and no germination (HB224, T 13 & P 4). Here, the GA3 test was able to differentiate Anjali from the rests of the genotypes studied (Flow chart 1).

The varied response of cotton genotypes to hydrogen peroxide has been observed in the present study. The genotypes studied were grouped as well-developed root and shoot (Suriya, T 13, LRA 5166, Surabhi & SVPR-2), well developed shoots and poorly developed roots (T 7 & Anjali), well developed roots with well-developed shoots and unopened leaflets (M 12), well developed roots and shoots (HB 224 & Supriya) and Ungerminated seeds (P 4). Here, the hydrogen peroxide test was able to differentiate M 12 and P 4 from the rests of the genotypes studied. The ferrous sulphate test also could not differentiate among the genotypes and the seed solution turned black.

Flow chart 1. Response of seeds of cotton genotypes to chemicals



CONCLUSION

The present study showed that potassium hydroxide test, sodium hydroxide soak test, GA_3 test, hydrogen peroxide soak test were useful for identification of cotton genotypes and were found to be useful in grouping cotton genotypes.

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