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Research Article

COMPARISON OF VARIOUS CHEMICAL TESTS FOR VARIETAL CHARACTERIZATION IN SUNFLOWER (*HELIANTHUS ANNUUS* L.)

***Sathisha C.S., Rajendra Prasad, S. Rame Gowda and Thimmegowda M.N.**
Department of Seed Science and Technology, National Seed Project,
University of Agricultural Sciences, G.K.V.K, Bangalore-65
**Author for Correspondence*

ABSTRACT
The sunflower genotypes viz., KBSH-1, TCSH-1, PKVSH-27, APSH-11 and DSH-1, their parental lines and the varieties, Morden, Surya, AKSF-9, CO-2, CO-3, CO-4, GAUSF-15, NDSH-15 and SS-56 were grouped on the basis of chemical tests (NaOH (2 &5%), KOH (2 &5%), GA_3 (25,50 &100 ppm), 2,4-D (5,10 & 15 ppm) and Metribuzin (0.03 ppm)) sensitivity test. The study revealed that these tests could be effectively used for varietal characterization and determining the varietal purity of sunflower in seed testing laboratories as the cultivars showed distinct response to these chemical tests.

Key Words: *Genotypes, Characterization, Parental Lines, Chemical Tests, Sunflower*

INTRODUCTION
The present trend of continuous release of sunflower genotypes from Central and State Varietal Release Committee has warranted developing techniques of varietal identification at the laboratory level particularly when the seeds have been submitted for seed purity analysis. Maintenance of genetic purity of varieties is of primary importance for preventing varietal deterioration during successive regeneration and for ensuring varietal performance at an expected level. The use of morphological traits in varietal identification and purity testing is time consuming and needs more area. Hence, there is a need for some quick tests for varietal purity testing in sunflower. The chemical tests reveal differences in seeds and seedlings different crop varieties (Agarwal, R.L. and Pawar, A, 1990). These tests do not much require virtually no technical expertise or training 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 has been carried out to study the response of sunflower genotypes to various chemical tests for effective utilization in varietal characterization and purity analysis.

MATERIALS AND METHODS
The experiment was carried out at the Seed Technology Research Unit, Gandhi Krishi Vignana Kendra, University of Agricultural Sciences, and Bangalore-65. The following tests were conducted.

NaOH Test
Three replicates of ten seeds each were soaked in 2 and 5 per cent NaOH solution for one hour and change in color of the solution was observed. The genotypes were grouped as dark brown and light brown based on change in color of solution (Chakraborty and Agarwal, 1989).

KOH Test
Ten seeds were soaked in 2 and 5 per cent KOH solution in three replicates for three hour and change in color of the solution was observed. The genotypes were grouped as dark brown and light brown (Chakraborty and Agarwal, 1989).

Seedling Growth Response to GA_3
Fifty seeds in each replication were soaked in 25, 50 and 100 ppm GA_3 and germinated as per ISTA (1999). Observations were recorded on shoot and root length at 10^{th} day. The percentage increase in shoot and root length over control was compared in each genotype (Agarwal, R.L. and Pawar, A., 1990 and Lee *et al.* 1992).

Seedling Growth Response to 2, 4-D

Fifty seeds in replications were soaked in 5, 10 and 15 ppm 2, 4-D and germinated as per ISTA (1999). Observations were recorded on 10^{th} day. The per cent decrease in shoot and root length was calculated (Lee *et al.,* 1992)

Metribuzin Sensitivity Test

50 Seeds were planted one centimeter deep and spaced two centimeter apart in the sand media and maintained under controlled photoperiod (24 h) and temperature (25 °C) during the test period. Nine day old seedlings were sprayed with 0.03 ppm Metribuzin (Hardcastle 1979, Wax *et al.* 1976 and Weese *et al.* 1989). Seedlings were evaluated daily for injury such as appearance of brown spots followed by desiccation and curling of leaves, leaf drops and death of plants and classifieds as:

More Sensitive:

Seedlings showed sign of injury after 9 days and are dead after 16 days after application.

Moderately Sensitive:

Seedlings showed sign of injury after 13 days and are dead after 32 days after application.

Least Sensitive:

Seedlings showed sign of injury after 20 days and are dead after 38 days after application.

RESULTS AND DISCUSSIONS

Both NaOH and KOH test at 2 and 5 per cent gave similar results in almost all the genotypes except CMS-2A, Surya, AKSF-9 and CMS-7-1A, where they responded as dark brown in KOH and Light brown in NaOH test. Out of twenty seven genotypes tested nine viz., 6D-1, RHA-272, CMS-2A, CMS-2B, AK-1R, Surya, AKSF-9, RHA-271 and GAUSUF-15 were grouped as light brown and remaining as dark brown in NaOH test (Table-1). NaOH and KOH tests in soybean Agarwal and Dadlani (1987) and Blackgram Chakrabarti and Agarwal (1989) were used to characterize cultivars

Based on the per cent increase in seedling growth genotypes 6D-1, NDSH-15, AKSF-9 and CMS-7-1B were grouped as high responsive (>20%), CMS-234A, CO-4, PKVSH-27, CMS-2A, CMS-2B and AK-1R as medium responsive (10-20%) and remaining as low responsive (<10%) for shoot length. Similarly based on increase in root length the genotypes CMS-234A, 6D-1, CO-3 and CMS-2A as high responsive, AK-1R, CMS-7-1B and GAUSUF-15 as medium responsive and the remaining as low responsive. Similar grouping of varieties was done by Chakrabarti and Agarwal (1990) in black gram, Agarwal and Pawar (1990) and Lee *et al.* (1992) in soybean.

Application of 2, 4-D has shown variation among the genotypes with respect to shoot and root length. The genotypes CMS-2A, APSH-11 and DSH-1 were grouped under tolerant since the shoot length decreased by 30 per cent over control. Based on root length all the genotypes were grouped under susceptible (30-60 %) and highly susceptible (>60 %) to 2, 4-D application (Table-2). The decrease in seedling growth among the genotypes may be due to the differential ethylene production upon application of 2, 4-D (Sundaru *et al.,* 1993). The use of growth regulators in the present study suggested that it could be used for broad grouping of genotypes rather than individual genotypes identification. Similar grouping based on the response to 2, 4-D was done by Agarwal and Pawar (1990) in soybean and Chakrabarti and Agarwal (1990) in black gram.

Among the 27 genotypes studied 14 showed increases in shoot length and 12 recorded decreased root lengths. Only six genotypes viz., APSH-11, KBSH-1, RHA-857, DSF-15A, DSF-15B and Morden showed increase in seedling growth (shoot and root length) (Figure -1). This differential activity was utilized for identifying genotypes. Similarly in 2, 4-D test individual genotypes were identified based on the per cent reduction over control in their root and shoot length (Figure -2). Genotypes APSH-11, DSH-1, CMS-7-1A and SS-56 were easily differentiated from genotypes Morden, AKSF-9 and CMS-234B based on root and shoot length.

Sunflower genotypes were grouped into two groups as most and moderately sensitive based on the response of seedlings to herbicide Metribuzin (Table 2). Genotypes 6D-1, RHA-272, Morden, NDSH-15,

DSH-1, DSF-15B, DSF-15A and GAUSUF-15 were grouped under moderately sensitive and remaining genotypes were grouped under most sensitive. None of the genotypes recorded least sensitivity to Metribuzin application. Similarly, the Metribuzin sensitivity test was used to characterize the soybean varieties by Hardcastle (1979), Wax *et al.* (1976) and Weese *et al.* (1989).

No single test contributes for successful identification. Hence, combination of these tests will be useful in characterization of sunflower genotypes. Thus, the chemical tests could be used as supplementary test for

Table 1: Response of sunflower genotypes to KOH and NaOH tests

S. No.	Genotypes	KOH test		NaOH test	
		2 per cent	5 per cent	2 per cent	5 per cent
1.	KBSH-1	Dark brown	Dark brown	Dark brown	Dark brown
2.	CMS-234 A	Dark brown	Dark brown	Dark brown	Dark brown
3.	CMS-234 B	Dark brown	Dark brown	Dark brown	Dark brown
4.	6 D-1	Light brown	Light brown	Light brown	Light brown
5.	TCSH-1	Dark brown	Dark brown	Dark brown	Dark brown
6.	RHA-272	Light brown	Light brown	Light brown	Light brown
7.	CO-2	Dark brown	Dark brown	Dark brown	Dark brown
8.	CO-3	Dark brown	Dark brown	Dark brown	Dark brown
9.	CO-4	Dark brown	Dark brown	Dark brown	Dark brown
10.	MORDEN	Dark brown	Dark brown	Dark brown	Dark brown
11.	NDSH-15	Dark brown	Dark brown	Dark brown	Dark brown
12.	SS-56	Dark brown	Dark brown	Dark brown	Dark brown
13.	PKVSH-27	Dark brown	Dark brown	Dark brown	Dark brown
14.	CMS-2A	Light brown	Light brown	Dark brown	Dark brown
15.	CMS-2B	Light brown	Light brown	Light brown	Light brown
16.	AK-1-R	Light brown	Light brown	Light brown	Light brown
17.	SURYA	Light brown	Light brown	Dark brown	Dark brown
18.	AKSF-9	Light brown	Light brown	Dark brown	Dark brown
19.	APSH-11	Dark brown	Dark brown	Dark brown	Dark brown
20.	CMS-7-1 A	Dark brown	Dark brown	Light brown	Light brown
21.	CMS-7-1 B	Dark brown	Dark brown	Dark brown	Dark brown
22.	RHA-271	Light brown	Light brown	Light brown	Light brown
23.	DSH-1	Dark brown	Dark brown	Dark brown	Dark brown
24.	DSF-15 B	Dark brown	Dark brown	Dark brown	Dark brown
25.	DSF-15 A	Dark brown	Dark brown	Dark brown	Dark brown
26.	RHA-857	Dark brown	Dark brown	Dark brown	Dark brown
27.	GAUSUF-15	Light brown	Light brown	Light brown	Light brown

Table 2: Grouping of sunflower genotypes based on their seedling growth response to GA_3, 2, 4-D and Metribuzin

Grouping GA_3 Test	High Responsive (>20 %)	Medium Responsive (10-20 %)	Low Responsive (<10 %)
Increase in shoot length	6 D-1, NDSH-15, AKSF-9, CMS-7-1B	CMS-234A, CO-4, PKVSH-27, CMS-2A, CMS-2B, AK-1R	KBSH-1, CMS-234B, TCSH-1, RHA-272, CO-2, CO-3, Morden, SS-56, Surya, APSH-11, CMS-7-1A, RHA-271, DSH-1, DSF-15B, DSF-15A, RHA-857, GAUSUF-15
Increase in root length	CMS-234A, 6 D-1, CO-3, CMS-2A	AK-1R, CMS-7-1B, GAUSUF-15	KBSH-1, CMS-234B, TCSH-1, RHA-272, CO-2, CO-4, Morden, NDSH-15, SS-56, PKVSH-27, CMS-2B, Surya, AKSF-9, APSH-11, CMS-7-1A, RHA-271, DSH-1, DSF-15B, DSF-15A, RHA-857
2,4-D test	**Tolerant (<30 %)**	**Susceptible (30-60 %)**	**Highly Susceptible (>60 %)**
Decrease in shoot length	CMS-2A, APSH-11, DSH-1	KBSH-1, CMS-234A, 6 D-1, TCSH-1, RHA-272, CO-2, CO-3, CO-4, NDSH-15, SS-56, PKVSH-27, CMS-2B, AK-1R, Surya, CMS-7-1A, RHA-271, DSF-15A, RHA-857, GAUSUF-15	CMS-234B, Morden, AKSF-9, CMS-7-1B, DSF-15B
Decrease in root length	-	CMS-234A, TCSH-1, CO-2, CO-3, SS-56, CMS-2A, AK-1R, Surya, APSH-11, CMS-7-1A, RHA-271, DSH-1	KBSH-1, CMS-234B, 6 D-1, RHA-272, CO-4, Morden, NDSH-15, PKVSH-27, CMS-2B, AKSF-9, CMS-7-1B, DSF-15B, DSF-15A, RHA-857
Metribuzin Test	**Least Sensitive**	**Most Sensitive**	**Moderately Sensitive**
Seedling sensitivity	-	KBSH-1, CMS-234A, CMS-234B, TCSH-1, CO-2, CO-3, CO-4, SS-56, PKVSH-27, CMS-2A, CMS-2B, AK-1R, Surya, AKSF-9, APSH-11, CMS-7-1A, CMS-7-1B, RHA-271, RHA-857	6 D-1, RHA-272, Morden, NDSH-15, DSF-15B, DSH-1, DSF-15A, GAUSUF-15

Varietal characterization and determination of the varietal purity in sunflower genotypes since these tests are reliable, simple, quick and cost effective.

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