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Chemical and Biochemical Characterization of Safflower (*Carthamus tinctorius*) Genotypes

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ABSTRACT: The present investigation entitled, Characterization of safflower genotypes by chemical and biochemical analysis was carried out at (Seed Technology Research Unit (STRU) laboratory) Department of Agricultural Botany and Department of Biochemistry, Mahatma Phule Krishi Vidyapeeth, Rahuri for testing quality of sixteen safflower genotypes. There was significant difference in the seed coat colour development due to NaOH test. The cultivars were grouped into three colour reaction groups as light brown (SSF-1307, Bhima, SSF-708, SSF-674, A-1, SSF-748, Phule kusuma), Brown (Nari-6, PBNS- 86, SSF-1371, PBNS-12, SSF- 733) and Dark brown (SSF-1240, Nari-57, SSF-658, PBNS-40) based on colour reaction to NaOH test. There was significant difference in total phenol content of different safflower genotypes. The highest phenol content was recorded in the genotype SSF- 748 (5.56 mg/g). All sixteen safflower genotypes under study showed no colour reaction (negative) for peroxidase test. The safflower genotypes showed significant difference to gibberellic acid test. The response of seedling of different safflower genotypes to GA_3, genotypes were grouped into very low response over control (SSF-1240, SSF-658, SSF-1307, SSF-748), low response over control (SSF-1371, SSF-674, Nari-6), medium response over control (SSF-733, PBNS-40, A-1, SSF-708, Nari-57) and high response over control (Bhima, Phule kusuma, PBNS-86, PBNS-12). The NaOH test, Phenol content and Gibberellic acid test can be used for characterization of safflower genotypes under study.

Keywords: Characterization, Gibberellic acid test, NaOH test, Phenol content, Safflower

Safflower (*Carthamus tinctorius* L.) is a member of Asteraceae family originated in the region spanning India, Afghanistan and Ethiopia. Safflower is highly branched, herbaceous, thistle like annual or winter annual, usually with many sharp spines on the leaves. The deep root system enables the plant to draw moisture and nutrients from a considerable depth, conferring on safflower the ability to survive in areas with little surface moisture [1]. The cultivated safflower is a diploid with 24 chromosomes and genome size of about 1.4 Gb [2]. Traditionally, it is grown for its seeds, flowers, fabric dyes, food coloring and for medicinal reasons [3]. In India, it is primarily grown for vegetable oil purpose. Safflower oil (*Carthamus tinctorius L.)* has a high content of polyunsaturated linoleic acid and tocopherol and is produced for nutritional as well as for medicinal uses [4]. Secondary metabolites are a subject of increasing research interest.Safflower oil contains high linoleic acid (>70%) (a polyunsaturated fatty acid), which is considered healthy as it reduces blood cholesterol level but has poor shelf life. High oleic safflower oil (>70%) is becoming more popular due to high stability and is highly preferred by the food industry. A natural dye called Carthamin extracted from its brilliantly colored flowers is used for coloring foods, cloths, preparation of cosmetics etc.

During the last decade, the global area under safflower cultivation ranged from 0.70 to 0.98 million ha and the production ranged from 0.53 to 0.83 million tonnes. India accounted for >30% (0.23 million ha) of area and >20 per cent (0.15 million tonnes) of global production. In India, safflower occupies seventh place among oilseed crops *viz*., groundnut, rapeseed and mustard, soybean, castor, sunflower, linseed, sesame and niger. It is grown over 3 lakh ha with a production of about 1.89 lakh tonnes and the productivity of about 630 kg/ha [5]. Maharashtra, Karnataka and Andhra Pradesh are the major safflower growing states in India. Many varieties of safflower are evolved by the breeders according to region climate. The genetic purity of these varieties needs to maintain during seed production. Many ways are followed for genetic purity testing.

Documentation of distinguishing characters of varieties is essential to carryout scientific seed production.

Unfortunately such complete information on distinguishing characters of different varieties is not available at one place. The cultivar identification has attained critical importance in national and international seed program. Safflower cultivar can be differentiated on the basis of effect of different chemical and biochemical reactions on seed and seedlings. The difference in chemical composition in oil content, phenol content can also be criteria for distinguishing the safflower varieties/ hybrids/ their parental lines from each other. Therefore, it was considered essential to record the necessary detailed information on characteristics of safflower cultivars. 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 safflower. The chemical tests reveal differences in seeds and seedlings different crop varieties [6]. 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.

MATERIALS AND METHODS

An experiment was carried out at Department of Agricultural Botany and Seed Technology Research Unit (STRU) laboratory, Department of Biochemistry, Mahatma Phule Krishi Vidyapeeth, Rahuri, during 2018-2019 to characterized safflower (*Carthamus tinctorius* L.) genotypes. The experiment consisted of sixteen genotypes, details are given in table 1.

A. Chemical Analysis

1. NaOH test

The NaOH reaction for seed coat colour was observed with 50 seeds of each genotype in four replications. The seeds were soaked in 5% NaOH solution at room temperature (25-30°C). The change in colour of seed coat was observed after one hour. The cultivars were categorised into three colour reaction groups as light brown, brown and dark brown [7].

2. Total phenol content

Estimation of phenols was carried out with Folin-Ciocalteu reagent method described by [8]. Phenols react with an oxidizing agent phosphomolybdate in Folin-Ciocalteu reagent under alkaline conditions and result in the formation of a blue coloured complex. The molybdate was measured at 650 nm calorimetrically.

Reagents

1. 80% ethanol
2. Folin-Ciocalteu reagent (FCR)
3. 20% Na_2CO_3
4. Standard (1mg/ml)**:**100mg catechol was dissolved in 100 ml of water diluted 10 times for a working standard.

Procedure

1. The (0.500g) leaf sample was weighed. The leaf sample was washed with sterile distilled water and blotted with filter paper and macerated in a pestle and mortar in five time volume with 80% ethanol.
2. Centrifuge the homogenate at 10,000 rpm for 20 min. and saved the supernatant.
3. Re-extract the residue two times with the volume of 80% ethanol.
4. The supernatant was evaporated to dryness.
5. The residue was dissolved in a known volume of distilled water (5ml).
6. The aliquots (0.1ml) were pipette out into test tubes.
7. The volume was made up to 3 ml with water (in each tube).
8. The Folin-Ciocalteu reagent (0.5 ml) was added.
9. After 3 min, 2ml of 20% Na_2CO_3 solution was added to each tube.
10. Mixed thoroughly and placed tubes in boiling water for exactly one min, cooled and absorbance was measured at 650 nm against a reagent blank.
11. Prepared a standard curve using different concentration of catechol.

Table 1. Sixteen Safflower genotypes

1. Nari-6	5. SSF-674	9. SSF-1371	13. SSF-708
2. SSF-658	6. SSF-1240	10. PBNS-86	14. Phule Kusuma
3. PBNS-40	7. SSF-748	11. SSF-1307	15. A-1
4. PBNS-12	8. SSF-733	12. Nari-57	16 Bhima

B. Biochemical Analysis

1. Peroxidase activity test

The seed coat was crushed and put in test tubes, then about ten drops of 0.5% guaiacol was added. After ten minutes one drop of 0.1% Hydrogen peroxide was added. One minute after adding the H_2O_2, the seed coat was observed for peroxidase activity. Observations was recorded for colour of seed coat as peroxidase positive indication by a reddish brown solution or peroxidase negative indication by a colourless solution in the test tube [9].

2. Seedling response to gibberellic acid (GA_3) test

Forty randomly selected seeds (10 seeds each in 4 replications) were placed on two layers on germination towel paper of 24 cm x 14 cm size moistened with GA_3 solution (25 ppm), sufficiently apart at the middle of the towel paper. Subsequently, they were covered with another sheet of GA_3 moistened towel paper towel and rolled along with untreated set as control. These rolled towels were then placed in seed germinator at 25°C for seven days, on 8thday the coleoptile length was measured in centimetres for all the ten seeds. The coleoptile length was calculated. The coleoptile, growth response to GA_3 was determined on the basis of per cent increase over control [9].

The varieties were classified into four categories *viz.,* very low, low, medium and high response increased over control.

RESULTS AND DISCUSSION

The chemical tests *viz.,* gibberellic acid (GA_3) test, peroxidase test, total phenol content and NaOH test were carried out separately. The results obtained from these studies are presented below.

A. Chemical Test Analysis

1. NaOH test

The standard NaOH test for seed coat reaction was carried out as per standard procedure. There was significant difference in the seed coat colour development due to NaOH test. According to this test, the cultivars were grouped into three colour reaction groups (Figure 1) as light brown (SSF-1307, Bhima, SSF-708, SSF-674, A-1, SSF-748, Phule kusuma), Brown (Nari-6, PBNS- 86, SSF-1371, PBNS-12, SSF- 733) and Dark brown (SSF-1240, Nari-57, SSF-658, PBNS-40). The different response of varieties to NaOH test may be due to some difference in chemical composition of the varieties. Similar type of test is also used in wheat for characterizing wheat genotypes. Genetically red wheat contains pigments, probably in the seed coat, that turn brown when exposed to NaOH [10]. These pigments are absent in genetically white wheat, so the change in color of white wheat to straw yellow after treatment with NaOH may be due to other compounds, possibly flavones and carotenoids [11], in the endosperm or seed coat that show through the transparent pericarp. Genetically white wheat cultivars that appear red likely have a reddish pigment in the endosperm or seed coat that either does not change color or becomes somewhat yellow upon treatment with NaOH.

Genetically red wheat that appears white may contain low concentrations of pigments that impart color to kernels other than the pigments that react with NaOH and turn brown. Because all genetically red wheat contain the critical pigments that turn brown upon treatment with

Figure 1. Response of different safflower genotypes to NaOH test

Table 2. Total phenol content of different safflower genotypes

Sr. No.	Name of varieties	Total phenol content (mg/g)	Sr. No.	Name of varieties	Total phenol content (mg/g)
1	Nari-6	3.13	9	SSF-1371	4.90
2	SSF-658	3.02	10	PBNS-86	3.42
3	PBNS-40	3.31	11	SSF-1307	5.23
4	PBNS-12	3.69	12	Nari-57	3.70
5	SSF-674	3.54	13	SSF-708	3.18
6	SSF-1240	3.33	14	Phule Kusuma	3.69
7	SSF-748	5.56	15	A-1	2.36
8	SSF-733	2.27	16	Bhima	3.34
	SEm(±)	0.018		CD (p=0.05)	0.052

NaOH, the NaOH test determines the genetic color class independent of visual appearance before soaking.

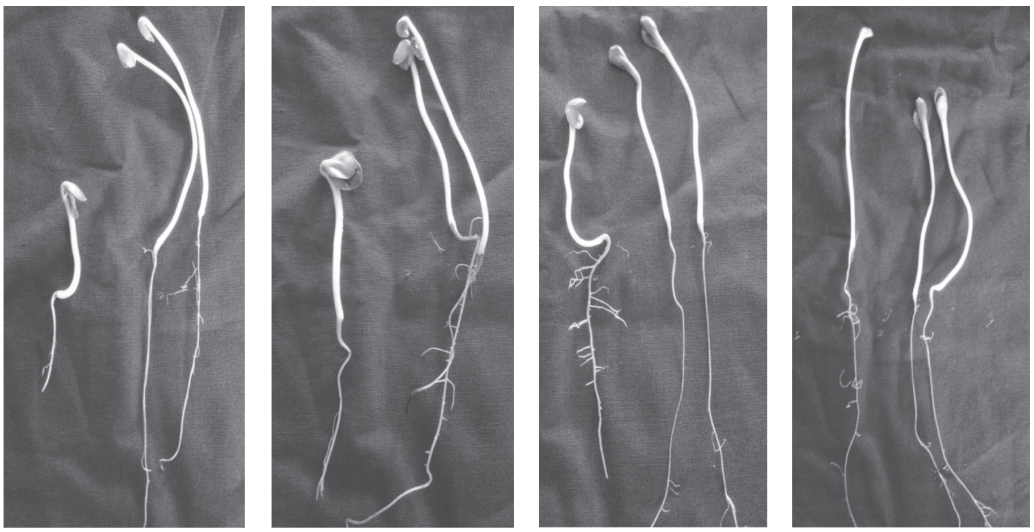
2. Total phenol content

The data on total phenol content of different safflower genotypes are presented in Table 2. The data revealed that there are significant differences in total phenol content of different safflower genotypes. The highest phenol content was recorded in the genotype SSF- 748 (5.56mg/g) where as the lowest phenol content in genotype SSF-733 (2.27mg/g). Safflower oils obtained from the seeds of plants of safflower cultivated were rich in phenolic constituents and demonstrated good antioxidant activity [12]. Thus, this oil, being rich in phenolic acids, could be a good source of natural antioxidants. Seed of safflower was good source for phenolic compounds with good antioxidant activity. The cause of the high antioxidant activity of safflower flower can possibly be due to including serotonins, flavonoids and lignans, compounds which are characteristic to possess antioxidant effects. These differences in chemical composition of safflower genotypes may help in differentiating the safflower genotypes.

B. Biochemical Test Analysis

1. Peroxidase test

The peroxidase test was carried out according to standard procedure. All the safflower genotypes under study showed no colour reaction (negative) to peroxidase test.



Very low response over control

SSF-1240, SSF-658, SSF-1307, SSF-748

Low response over control

SSF-1371, SSF-674, Nari-6

Medium response over control

SSF-733, PBNS-40, A-1, SSF-708, Nari-57)

High response over control

Bhima , Phule kusuma, PBNS-86, PBNS-12)

Figure 2. Response of different safflower genotypes to gibberellic test

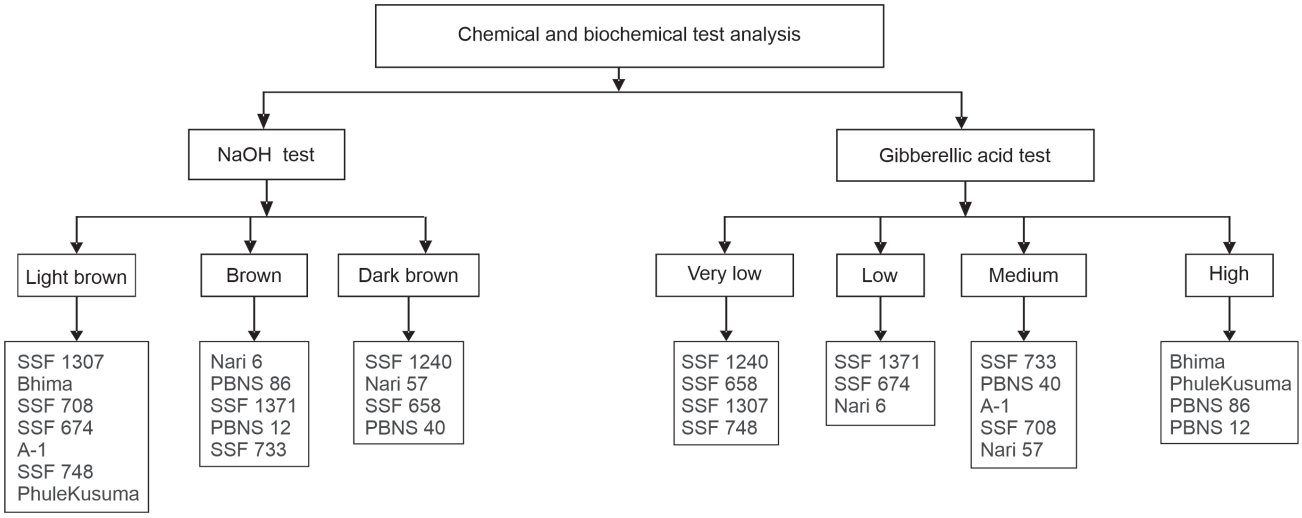


Figure 3. Keys based on the analysis of chemical and biochemical tests

The peroxidase test had been used for variety testing extensively in soybean. It has been used as a qualitative test i.e. peroxidase activity is present or absent done by visual observation.

2. Gibberellic acid (GA_3) test

Safflower genotypes showed significant difference to gibberellic acid test. The response of seedling of different safflower genotypes to GA_3, genotypes were grouped (Figure 2) into very low response over control (SSF-1240, SSF-658, SSF-1307, SSF-748), low response over control (SSF-1371, SSF-674, Nari-6), medium response over control (SSF-733, PBNS-40, A-1, SSF-708, Nari-57) and high response over control (Bhima, Phule kusuma, PBNS-86, PBNS-12).The difference in coleoptile length may be due to differences in genetic makeup of genotypes and selective response to the growth regulator [13].

CONCLUSION

Characterization key was developed on the basis of chemical and biochemical analysis (Figure 3) and it could be deduced that NaOH test grouped safflower cultivars into different colour groups *viz.,* light brown (SSF-1307, Bhima, SSF-708, SSF-674, A-1, SSF-748, Phule kusuma), Brown (Nari-6, PBNS- 86, SSF-1371, PBNS-12, SSF- 733), and Dark brown (SSF-1240, Nari-57, SSF-658, PBNS-40) and proved useful for characterization of safflower cultivars. The highest phenol content was noticed in the genotype SSF-748 (5.56 mg/g) and lowest in genotype SSF-733 (2.27 mg/g). Safflower oils obtained from the seeds of plants of safflower were rich in phenolic constituents and had good antioxidant activity. All the safflower genotypes did not showed any response to peroxidase test.

The gibberellic acid (GA_3) test had categorized safflower cultivars into very low response over control (SSF-1240, SSF-658, SSF-1307, SSF-748), low response over control (SSF-1371, SSF-674, Nari-6), medium response over control (SSF-733, PBNS-40, A-1, SSF-708, Nari-57) and high response over control (Bhima, Phule kusuma, PBNS-86, PBNS-12). It was found useful for characterizing different safflower cultivars.

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