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CHEMICAL TESTS FOR IDENTIFICATION AND CHARACTERIZATION OF TOMATO CULTIVARS

ABSTRACT

Various chemical tests are being used to reveal chemical differences among the seeds or seedlings of different cultivars. Study to characterize and identify 24 tomato cultivars based on chemical test chemicals viz., Standard phenol test, Modified phenol test, NaOH test, KOH test and Seedling growth response to added chemicals revealed that, most of the cultivars studied were distinct from other cultivars. No single chemical test could distinguish all the varieties. However, distinguishable chemical characteristics were used to develop the keys for identification of each and every cultivar and all the cultivars were distinguished based on these identification keys.

Key words: chemical tests, tomato, varietal characterization

INTRODUCTION

Tomato (*Lycopersicon esculentum* L.) is the world's largest vegetable crop and known as productive and protective food because of its special nutritive value and wide spread production. Although it was introduced to India in the 18th century, its commercial cultivation began towards the end of last century. It is economically important for its edible fruits and preserved products like ketch-up, sauce, chutney, soup, paste, puree etc. Tomato is a rich source of minerals, vitamins and organic acids, essential amino acids and dietary fibers. It is also rich source of vitamin

A and C, it contains minerals like iron, phosphorus and pigments lycopene and beta-carotene.

At present, large number of varieties and hybrids with special regard to yield, fruit quality, resistant to biotic and abiotic stresses are under cultivation throughout the country. In recent years public institutions and private companies introduces one after the other hybrids/varieties for commercial cultivation. Therefore, the discrimination of tomato varieties, especially by examination of the seed is increasingly important in order to protect the breeders and farmers rights (Wang *et al.*, 2000) and to ensure genetic purity or genuineness of variety which is most important characteristics of a quality seed.

The most common method of varietal identification is grow out test (Arus *et al.*, 1982), which is based on the morphological markers, has many disadvantages such as interaction with environment, high cost and lengthy time required, low polymorphism in closely related varieties which were bred by using elite lines, to name but a few. Recent discoveries in the area of biochemistry and molecular biology have enabled seed scientist to utilize new techniques for cultivar identification to augment existing traditional methods. Proteins, isozymes and DNA markers are independent of environmental factors, these markers have been used to characterize (Tanksley *et al.*, 1992; Lucchese *et al.*, 1999, Vishwanath *et al.*, 2010) However, these requires high investment cost and sophisticated lab facility and skilled personnel. Therefore it is necessary to find simple and cost effective techniques for characterization and identification of tomato cultivars.

Reaction of seeds or seedlings to different chemicals is based on seed composition; this property has been used by several researchers to characterize many crop cultivars (Papp *et al.*, 1997, Vanangamudi *et al.*, 1998, McDonald Jr., 1985, Punia *et al.*, 2002, Patil *et al.*, 2006; Nethra *et al.*, 2007). There are several tests for varietal identification and the important tests are the soybean seed coat peroxidase test, oat seed fluorescence test, KOH-bleach test for sorghum, HCl tests for oats, the phenol test for wheat, *Poa pratensis* and *Lolium perenne*, the NaOH test to distinguish red and white wheat and rice varieties, the ammonia fluorescence test to distinguish *Festuca rubra* and *F. longifolia*, CuSO₄-ammonia test to distinguish *Melilotus officinalis* and *M. alba*, and the fluorescence test for *Lolium perenne* and *L. multiflorum* (ISTA, 1993; Mc Donald- Jr., 1985). However, among rapid chemical tests no single test was tried for tomato cultivar identification. Hence, different chemicals were used to reveal chemical reaction on seed constituents for characterization and identification of tomato cultivars.

MATERIALS AND METHOD

Seed samples of twenty four cultivars which are under cultivation in the state were collected from public and private organizations (Table 1) and subjected for different chemical tests .

Cultivarsoftomatousedforvarietalcharacterization

Table1

Sl No.	Cultivar	Developed institute/ company	Sl No.	Cultivar	Developed institute/ company
1.	Arka Alok	IIHR	13.	Nandi	UASB
2.	Arka Vikas	IIHR	14.	Sankranthi	UASB
3.	Arka Ahuti	IIHR	15.	Vybhav	UASB
4.	Arka Ashish	IIHR	16.	NS – 2535	Namdhari Seeds
5.	Arka Abha	IIHR	17.	Mruthyunjaya –2	Sasya Seeds
6.	Arka Megali	IIHR	18.	US –618	U.S. Agriseeds
7.	Arka Saurab	IIHR	19.	J.K. Desi	J.K. Agrigenetics
8.	Arka Shresta	IIHR	20.	J.K. Asha	J.K. Agrigenetics
9.	Arka Abijeet	IIHR	21.	Ronco	Bejo Seeds
10.	Pusa Ruby	IARI	22.	A -32/63	Indosem Seeds
11.	Pusa Early Dwarf	IARI	23.	128/M 131	Indosem Seeds
12.	PKM –1	TNAU	24.	M-03/868	Indosem Seeds

Standard phenol test:

Two hundred (50×4) seeds were presoaked in distilled water for 24 h at $25 \pm 1^\circ\text{C}$. Then they were transferred on to two layers of Whatman No.1 filter paper saturated with one per cent phenol solution. The petri dishes were covered and incubated at $25 \pm 1^\circ\text{C}$ 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 as No change in colour, Light brown, Brown, Dark brown, Light grey, Grey and Dark grey.

Modified phenol test

Modified phenol test was conducted similar to standard phenol test except that seeds were soaked either in 0.5 per cent CuSO_4 or one per cent FeSO_4 solution for 24 h instead of distilled water. Colour reaction was noted after 48 h of incubation and the cultivars were classified based on colouration of seed coat into different categories as No colour change, Light brown, Brown, Dark brown, Light grey, Grey and Dark grey.

NaOH test

Seeds were soaked in two per cent NaOH solution for one hour and thereafter change in colour of the solution was observed. Based on the intensity of the colour reaction, the genotypes were classified into two groups viz., no change in colour, yellow and light yellow.

KOH test

Seeds were soaked in four per cent KOH solution for three hours and thereafter change in colour of the solution was observed. Based on the intensity of the colour reaction, the genotypes were classified into two groups viz., no change in colour and reddish brown.

Seedling growth response to added chemicals

Seedling growth response to GA₃ application: The increase in shoot length, root length and primary leaf length due to exogenous application of GA₃ was measured. The seeds (100 × 4) were soaked in 50 ppm GA₃ solutions for 24 h and then germinated in rolled towels at 25 ± 1°C as per ISTA (1996). Twenty five seedlings were selected randomly and growth response was measured at 14th day of germination in terms of per cent increase in shoot length and root length over control. The mean of increased seedling length was determined and the cultivars were grouped into five categories (Agarwal and Pawar, 1990) based per cent increase over control viz., Very low response (< 25%), Low response type (25 to 50%), Medium response type (50 to 75%), High response type (75 to 100%) and Very high response type (> 100%) .

Seedling growth response to kinetin application: The increase in shoot length, root length and primary leaf length due to exogenous application of kinetin was measured. The seeds (100 × 4) were soaked in 50 ppm kinetin solutions for 24 hours and then germinated in rolled towels at 25 ± 1°C as per ISTA (1996). Twenty five seedlings were selected randomly and growth response was measured at 14th day of germination in terms of per cent increase in shoot length and root length over control. The mean of increased seedling length was determined and the genotypes were grouped into five categories according to Agarwal and Pawar (1990) based per cent increase over control viz., Very low response (< 25%), Low response type (25 to 50%), Medium response type (50 to 75%), High response type (75 to 100%) and Very high response type (> 100%).

Seedling growth response to 2,4-D application: The reduction in seedling growth due to exogenous application of 2,4-D was measured. The seeds (100 × 4) were soaked in 10 ppm of 2,4-D solutions for 24 h and then tested for germination in rolled towels at 25 ± 1°C as per ISTA (1996). Growth response was measured on seventh day in terms of per cent decrease in shoot length over control. The reduction in mean seedling length was expressed and based on growth inhibition, the genotypes were classified into three categories (Agarwal and Pawar 1990) viz., Highly resistant type (< 30%), Medium resistant type (30 to 60%) and Low resistant type (> 60%).

The data was analyzed statistically by following Completely Randomized Design (CRD) and adopting “Fischer’s Analysis of Variance Technique” critical difference values were calculated at 5 per cent probability level

(where “F” test was significant). Dunnett’s “t” test was then performed to compare control with all other treatments (Panse and Sukhatme, 1967).

RESULTS AND DISCUSSION

Chemical tests

Seeds constituents vary between the cultivars. The reaction of these constituents with chemicals resulted in different reaction. Various biochemical tests are being used to reveal chemical differences among the seeds or seedlings of different cultivars. They require virtually no technical expertise or training and can be completed in a relatively short time. The results of these test are usually distinct, easily interpreted and helps in distinguishing or grouping of cultivars. Phenol colour reaction depends on quality and quantity of oxidative enzymes present in seeds (Walla, 1965). Whereas, monophenol oxidase is extremely localized in seeds, even though it is present in all other plant parts (Takahashi and Hamza, 1983). Phenol colour reaction is an index of polyphenol oxidase activity, has been utilized to distinguish the crop varieties (Joshi and Banerjee, 1970, Abrol and Uperty, 1972, Chauhan and Nanda, 1984, Sparks *et al.*, 1985).

An attempt to characterize 24 tomato cultivars was made based on the phenol colour reaction test. Cultivars Arka Vikas, Arka Ahuti, Arka Megali, Arka Saurab, Nandi, Sankranthi, Vybav, NS-2535, JK Desi, 128/M 131 did not respond to phenol reaction. Among reacted cultivars Arka Alok, Arka Shresta, Arka Ashish, Pusa Early Dwarf, US-618, JK Asha, A 32/63, M-03/868, Mruthunjaya-2, Ronco showed light brown and Arka Abha, Arka Abijeet, Pusa Ruby showed brown colour. Only PKM-1 was distinct from other cultivars by its dark grey colour and could be used as marker to identify from rest of the cultivars (Fig 1).

Fig1. Reaction of seed to Standard Phenol Test

Colour reaction of phenol was enhanced by using $\text{Cu}^{++}/\text{Fe}^{++}$ as a catalyst by using CuSO_4 and FeSO_4 . Based on this the cultivars were classified into seven

groups as no colour change, light brown, brown, dark brown, light grey, grey and dark grey. Arka Saurab was distinct by its no colour reaction with either CuSO_4 or FeSO_4 , cultivar Arka Vikas by its dark grey with Fe^{++} ions. Hence, based on colour reaction with phenol and modified phenol test genotypes can be classified into different groups and phenol test with FeSO_4 found to be better in distinguishing the cultivars (Fig 2). The presence of metallic ions Fe^{++} and Cu^{++} in modified phenol test enhances the phenol colour reaction since it is an enzymatic reaction and these ions act as catalyst (Banerjee and Chandra, 1977), which was confirmed by Gupta and Agarwal (1988), Agarwal and Karki (1989). Further, phenol colour reaction of seeds seems to be due to the difference in the genetic background, presumably concerning the enzyme system (Takahashi and Hamza, 1983). The genotypes were grouped based on phenol colour reaction test in several crop plants by Meshram and Ratiangdale (1988), Wang and Shen (1992), Vnanagmudi *et al.*, (1988), Panwar and Ram (1988) in Wheat, Jaiswal and Agarwal (1995) and Nethra *et al.*, (2007) in rice. In the present study, additional three more colour categories could be observed viz., light grey, grey and dark grey which was not reported by earlier studies on any crops. The present findings revealed that phenol and modified phenol test with CuSO_4 and FeSO_4 could be used as simple, quick and cheap method for identification of some studied cultivars and grouping of most of the cultivars.

Fig 2. Reaction of seed to Modified Phenol Test with CuSO_4

Based on the colour reaction to NaOH and KOH the cultivars were grouped into three and four categories respectively. Based on NaOH test cultivars were categorized into no colour change, light yellow and dark yellow. Seventeen cultivars did not respond to NaOH test by showing no colour change. However, cultivar A 32/63 was distinct from other cultivars by its dark yellow stain. Based on KOH test cultivars, were categorized into four categories viz., no colour change, light yellow, dark yellow and wine red. Nine cultivars did not respond to KOH test. Cultivar NS-2535 was distinct from rest of the cultivars by its wine red staining (Fig 3). The difference in colour change to either NaOH/KOH was due to difference in seed constituents. One extra category dark yellow was found and was utilized for grouping of genotypes than earlier categories which were reported by many

scientists (Vanangamudi *et al.*, 1998, Nethra *et al.*, 2007 in rice Mc Donald Jr., 1985, Punia *et al.*, 2002 in wheat, Patil *et al.*, 2006 in safflower, Papp *et al.*, 1997 in *Brassica spp.*). However, in present study wine red category was not found in NaOH test but found in KOH test.

Fig 3. Reaction of seed to KOH test

Seedling response to added chemicals

The growth response of cultivars to nutrients or growth regulators is mainly governed by its genetic make up and expression of genes at specific condition. The effect of growth regulators on seedling growth behavior has been used in characterizing cultivars of several crops. (Nethra *et al.*, 2007 in rice, Agarwal and Sharma, 1989, in bean. Patil *et al.*, 2006 in safflower).

Based on response to GA_3 , cultivars were grouped into five categories viz., very low (<25%), low (25-50%), medium (50-75%), high (75-100%) and very high (>100%). Only cultivar NS-2535 was distinct by its very high (156.00%) response to GA_3 followed by JK Desi (97.49%). Very low response (11.61%) was noticed in Arka Megali. All the cultivars were grouped based on response to GA_3 . Similar results were reported by Goyal and Baijal (1980) who opined that differences might be due to increased RNA activity or due to differences in cell division (Terao, 1986).

Cultivar Arka Alok was distinct from rest of the cultivars by its very low response (<25%) to 50 ppm kinetin. Arka Ahuti, Arks Saurab and PKM-1 were also distinct by their medium response (50-75%) to kinetin. Similarly, Mruthunjaya-2, JK Asha, Ronco, 128/M 131 by their high response (75-

100%). Rest of the cultivars were categorized either in to low (25-50%) or very high (>100%). Cultivar Pusa Ruby showed maximum response (136.60%) followed by Arka Abijeet (108.33%) (Table 2). The increase in growth might be due to amplified cell division during early phases of seed germination (Prasad and Roy, 1999 in pigeon pea, Nethra *et al.*, 2006 in rice).

The highest reduction in growth due to exogenous application of 2,4-D was observed in Mruthunjayua-2 (157.55%) followed by NS-2535 (109.60%) and least in Pusa Ruby (30.92%). Most of the cultivars were grouped under highly susceptible (>60%) category except cultivars Arka Alok, Arka Vikas, Arka Ashish, Arka Abha, Arka Saurab, Pusa Ruby and M-03/868 which showed susceptible nature (Table.2). However, no cultivar was tolerant to 2,4-D. The differences in reduction in seedling growth among genotypes might be due to differential ethylene production upon application of 2,4-D (Sundaru *et al.*, 1983) which governed signal transduction and expression of specific gene for external stimuli which is further controlled by genetic make up of genotype.

Table 2

Response of cultivars of tomato to different growth regulators.

Sl No.	Genotypes	Control	GA ₃ [50 ppm]	Kinetin [50 ppm]	2,4-D [10 ppm]
1.	Arka Alok	13.89	12.52	11.79	51.07
2.	Arka Vikas	14.66	39.61	150.0	31.50
3.	Arka Ahuti	14.54	24.59	50.10	73.38
4.	Arka Ashish	14.67	39.11	30.55	41.11
5.	Arka Abha	15.89	24.12	38.46	48.91
6.	Arka Megali	14.56	11.61	43.11	62.91
7.	A. Saurab	14.89	24.79	56.11	52.12
8.	Arka Shresta	13.99	67.27	103.24	81.91
9.	Arka Abijeet	14.00	62.72	108.33	81.64
10.	Pusa Ruby	14.69	10.00	136.60	30.92
11.	Pusa Early Dwarf	14.89	13.66	38.46	60.96
12.	PKM-1	14.87	42.22	51.23	71.12
13.	Nandi	14.68	43.90	42.55	76.18
14.	Sankranthi	14.84	28.18	43.41	74.85
15.	Vybhav	15.87	53.08	67.74	77.13
16.	NS-2535	17.00	156.00	115.18	109.60

Table 2

Continued					
Sl No.	Genotypes	Control	GA ₃ [50 ppm]	Kinetin [50 ppm]	2,4-D [10 ppm]
17.	Mruthyanjaya-2	15.01	82.00	89.19	157.55
18.	US -618	17.56	88.04	131.1	82.62
19.	JK Desi	16.89	97.49	1.0.21	85.83
20.	JK Asha	17.06	89.19	99.11	87.45
21.	Ronco	15.11	86.36	89.28	74.96
22.	A 32/63	17.16	18.33	42.33	80.00
23.	128/M 131	15.00	30.78	82.05	39.72
24.	M-03/868	13.05	43.12	45.43	57.33
SEm±		0.961	3.525	6.86	4.83
CD (p=0.05)		2.693	9.88	19.36	13.61

Through lone chemical test could not able to differentiate all the cultivars. However, distinguishable chemical characteristics were used to develop the keys for identification of each and every cultivar and all the cultivars were distinguished based on these identification keys. (Fig 4). Hence, these chemical tests are simple, quick, cost effective and virtually require no expertise and could be employed for varietal identification and characterization of tomato cultivars.

Fig 4. Identification Key based on chemical tests in tomato cultivars

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