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Characterization of Lentil Varieties based on Morphological and Biochemical Tests at Seed and Seedling Stages

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ABSTRACT Expressions of four morphological and reaction to six biochemical tests were performed on 25 varieties of lentil to observe distinguishing, uniform and stable expressions over three generations. Expression of seed coat colour, testa mottling and seedling pigmentation are designated as the most stable, uniform and distinguishable traits. Expression of the seed size is not stable for medium and bold seeded varieties. Among the biochemical tests performed for verification of varieties, use of phenol colour, KOH-bleach, HCI test and response of GA_3 on root and shoot elongation are advocated.

Key words: Genetic purity, seed, ODV, biochemical test, lentil

Lentil [Lens culinaris (Medik)], is the second most important winter pulse crop of India [1]. For exploitation of genetic potential of a variety used of pure seed at genetic level is pre-requisite. For maintenance of genetic purity of varieties during seed production and certification there is an urgent need for documentation of diagnostic features of varieties with their accurate identification keys (On comparative basis), with features of distinctness. Testing of genetic purity at seed level requires detailed information about the expression of morphological traits at seed and seedling stages.

Lentil has tremendous variability for seed size [2] and is classified as macrosperma (100-seed weight > 2.5 g) and microsperma (100-seed weight < 2.5 g). 100-seed weight ranges from 1.10 to 8.0 g [3-8]. Lentil varieties have been categorized as grey, brown, black, pink [9-12]; green, greenish brown and light red speckled with black [4, 5, 13] based on seed coat colour. Based on mottling, uniform black, beige and mottled categories have been reported [10-12, 14]. Cotyledon colour of lentil genotypes have been reported as red, orange, yellow, green and bleaching to yellow [4, 5, 12, 13, 15, 16]. Seedling of lentil varieties has been categorized as, of purple and green epicotyl, based on pigmentation [9, 12, 17]. Not much distinctness is available on seed. Therefore, biochemical tests may be used to test the genetic purity. The present investigation was undertaken to characterize the varieties at seed i.e., metabolically stable stage to judge the varietal purity prior to sowing.

MATERIALS AND METHODS

Seeds of all the 25 varieties of lentil in active seed commerce were obtained from Indian Institute of Pulses Research, Kanpur and tested for expression of morphological traits and reaction to biochemical tests at seed and seedling levels during rabi 2003-2004 and 2004-2005 at JNKVV, Jabalpur. Randomly selected seeds were observed for distinct expressions of seed size (100-seed weight with 10-12% moisture content); seed coat colour (visual observation in daylight); testa mottling (magnification of 5X) and cotyledon colour. The following biochemical tests recommended for different crops were performed to verify the stable reactions for confirmation of genetic purity.

Peroxidase activity [8]; phenol colour [19]; HCl [20]; KOH [21]; potassium hydroxide-bleach [22] and growth response of seedling to GA_3 solution [23] were performed to observe distinguishable response of the varieties based on biochemical tests.

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RESULTS AND DISCUSSION

Characterization based on seed morphology

To characterize the varieties at seed and seedling levels, 10 characters were studied. At seed level, seed coat colour is a distinguishing character, which is controlled by two pairs of genes [24, 25], whereas monogenic control of seed coat colour was reported by Ladizinsky [26, 27]. On the basis of seed coat colour, all the varieties were categorized as grey, brown, pink and black. The trait may be used as distinct, stable and uniform for verification of varieties (Table 1).

Testa mottling is another stable distinguishing character controlled by two pairs of genes [14]. Only two types of classes were formed based on testa mottling i.e., presence of mottling and absence of testa mottling. Seed coat colour and testa mottling are found to be the most stable and uniform traits for verification of genetic purity at seed level as their expression are least influenced by environment. Hence, the use of testa mottling with seed coat colour as distinguishable, uniform and stable trait is advocated.

No variation was observed for cotyledon colour as all the varieties were of orange cotyledon. However, orange, yellow and green cotyledons are reported [9-12]. Monogenic inheritance [28, 29] with distinctness between orange and yellow cotyledons ensures its use as distinct, stable and uniform character in future. Most of the varieties in India are developed in the background of microsperma type and the varieties are classified based on seed size. Therefore, expression of the trait is not found uniform for medium and bold seeded varieties as they may have seeds of all the three categories. Hence, it should be considered with the help of stable traits. Varieties were categorized as purple and green on the basis of anthocyanin pigmentation at seedling stage. The use of this trait as distinguishable, stable and uniform is advocated, as it is monogenically controlled [26, 30, 31].

Characterization based on biochemical tests

Many morphological descriptors used to establish distinctness are multigenie and quantitative in nature, their expression may be altered by environmental factors. Moreover, in some species,

Table 1. Categorization of lentil varieties based on seed characteristics

Seed size			
Small (<2.1 g)	**Medium (2.1 to 2.6 g)**	**Bold (>2.6 g)**	
Asha, L 4147, LL 147, LL 56, I.L 699, PL 4, PL 406, PL 639, Ranjan, VL 1, VL 4, VL 103	DPL 15, IPL 81, JL 1, JL 3, K 75, L 4076, LH 84-6, NDL 1, PL 234, PL 77-12, Subrita	DPL 62, PL 5	
Seed coat colour			
Grey	**Brown**	**Pink**	**Black**
Asha, DPL 15, DPL 62, IPL 81, JL 1, K 75, L 4076, L 4147, LH 84-6, NDL 1, PL 234, PL 406, PL 5, PL 639, PL 77-12, VL 103	LL 147, LL 56, LL 699, PL 4	JL 3, Ranjan, Subrita	VL 1, VL 4
Testa mottling			
Presence of testa mottling	**Absence of testa mottling**		
Asha, DPL 15, DPL 62, IPL 81, JL 1, JL 3, K 75, L 4076, L 4147, LH 84-6, LL 56, LL 699, LL 147, LL 639, NDL 1, PL 5, PL 77-12, PL 234, PL 639, PL 406, Ranjan, Subrita, VL 4, VL 103	VL 1		
Cotyledon colour			
Orange	**Yellow**		
Asha, DPL 15, DPL 62, IPL 81, JL 1, JL 3, K 75, L 4076, L 4147, LH 84-6, LL 56, LL 699, LL 147, LL 639, NDL 1, PL 5, PL 77-12, PL 234, PL 639, PL 406, Ranjan, Subrita, VL 1, VL 4, VL 103	Nil		

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the number of descriptors is limited or is no longer sufficient for identification of all the varieties. Identification of varieties through biochemical methods has the advantage of being rapid, reliable, stable and with high discriminatory power.

Phenol colour test was performed based on the activity of phenol oxidase enzyme present in seed testa. As a result of enzymatic oxidation of phenol, dark insoluble pigments i.e., melanin are formed. On the basis of amount and kinds of various phenol oxidase enzymes, varieties (Table 2) were categorized as non-responsive (DPL 62); light brown (LL 56, LL 147 and VL 103); brown (JL 1, L 4147, LH 84-6, LL 699, PL 5, PL 234, PL 406, PL 639, Subrita and VL 4) and black (all the remaining varieties). Black, brown and light brown colouration of seeds confirms the high, medium and low activity of phenol oxidase enzyme, respectively. The use of this test is advocated for characterization of varieties. The dark pigment in testa, which has been identified as tannic acid forms the basis for discrimination of lentil varieties by reaction with KOH-bleach. On the basis of colour developed on seeds, varieties were categorized as light brown (JL 3, Ranjan, Subrita, VL 1 and VL 4), brown (LL 56, LL 147, LL 699) and dark brown (all the remaining varieties). It was observed that the variety with brown seed coat mainly produces dark brown colour after reaction with KOH-bleach, whereas, variety with black or pink seed coat colour turns light brown after reaction with KOH-bleach. Grey seeded varieties turn brown after reaction. Presence of peroxidase enzyme in seeds of crops from family leguminoceae is under genetic control, therefore varieties may be verified with its reaction. On the basis of activity of peroxidase enzyme lentil varieties were categorized as low with very light brown (JL 1 and LL 147), medium with light brown (remaining varieties) and high with brown colour (K 75, PL 5, VL 1, VL 4 and VL 103). A distinguishing chemical marker of soybean [18] is not found as an important marker for discrimination of lentil varieties because of limited activity of the enzyme. No variation among the varieties was observed for KOH test as all the varieties had positive reaction with the development of deep red solution.

Based on intensity of colour developed on seeds with the reaction of HCl, varieties are categorized as light brown, brown and dark brown. Light brown colour is developed on seeds of variety, whereas, dark brown colour is developed on reaction with remaining varieties produce brown colour. HCl test was originally adopted to discriminate the varieties of oat at seed level [20]. Lentil varieties have been categorized as light brown (Asha), brown (all the remaining varieties) and dark brown (VL 1 and VL 4) on reaction with HCl. However, with passage of time, light brown and brown varieties developed dark brown solution of HCl, so observations should be recorded just after the reaction.

The response of GA_3 on root and shoot elongation at 20 ppm and 30 ppm concentration was observed. Very few varieties were found to be responsive at 20 ppm (L 4147) and 30 ppm (LL 56 & IPL 81) for shoot elongation. Varieties DPL 62, IPL 81, JL 3, LL 56, LL 699, NDL 1 and PL 4 responded at 20 ppm GA_3 solution and PL 234 responded at 30 ppm for root elongation. The test is found effective for discrimination of lentil varieties.

Except seed size, expression of the observed morphological and biochemical traits on seed was found to be uniform and stable before sowing and after harvesting for all the varieties in both the years, therefore, all the varieties are considered as stable for the expression of these traits (Table 3). Expression of the seed size is not found stable for medium and bold seeded varieties as they have seeds of all the three categories. Few off-types were recorded in variety PL 5 (cotyledon colour and reaction to phenol test) and NDL 1 (epicotyl colour).

Key for verification of lentil varieties at seed level

Based on seed size varieties were categorized (Fig. 1) as small (Asha, L 4147, LL 56, LL 147, LL 699, PL 4, PL 406, PL 639, Ranjan, VL 1, VL 4, VL 103); medium (DPL 15, IPL 81, JL 1, JL 3, K 75, L 4076, LH 84-6, NDL 1, PL 77-12, PL 234, Subrita); and bold (DPL 62, PL 5). Small seeded varieties are further categorized on the basis of seed coat colour as, grey (Asha, L 4147, PL 406, PL 639, VL 103); brown (LL 56, LL 147, LL 699, PL 4); pink (Ranjan); and black (VL 1, VL 4). Varieties with grey seeds are classified on the basis of phenol test as black (Asha); brown (L 4147, PL 406, PL

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Table 2. Categorization of lentil varieties based on biochemical tests

Phenol colour test			
Black	**Brown**	**Light brown**	**No reaction**
Asha, DPL 15, IPL 81, JL 3, K 75, L 4076, NDL 1, PL 4, PL 77-12, Ranjan, VL 1	JL 1, L 4147, LH 84-6, LL 699, PL 406, PL 234, PL 5, PL 639, Subrita, VL 4	LL 56, LL 147, VL 103	DPL 62

KOH-bleach test		
Light brown	**Brown**	**Dark brown**
JL 3, Ranjan, Subrita, VL 1, VL 4	Asha, DPL 15, DPL 62, IPL 81, JL 1, K 75, L 4076, L 4147, LH 84-6, NDL 1, PL 4, PL 5, PL 234, PL 639, PL 77-12, PL 406, VL 103	LL 699, LL 56, LL 147

Peroxidase activity test		
Very light brown	**Light brown**	**Brown**
JL 1, LL 147	Asha, DPL 15, DPL 62, IPL 81, JL 3, L 4076, L 4147, LH 84-6, LL 56, LL 699, NDL 1, PL 406, PL 234, PL 4, PL 639, PL 77-12, Ranjan, Subrita	K75, PL 5, VL1, VL 4, VL 103

HCI test		
Light brown	**Brown**	**Dark brown**
Asha	DPL 15, DPL 62, IPL 81, JL 1, JL 3, K 75, L 4076, L 4147, LH 84-6, LL 699, LL 4147, LL 56, NDL 1, PL 406, PL 234, PL 4, PL 5, PL 639, PL 77-12, Ranjan, Subrita, VL 103	VL 1, VL 4

Response to GA_3 for shoot elongation		
Non responsive	**Responsive to 20 ppm**	**Responsive to 30 ppm**
Asha, DPL 15, DPL 62, JL 3, JL 1, K 75, L 4076, LH 84-6, LL 147, LL 699, NDL 1, PL 234, PL 4, PL 406, PL 5, PL 639, PL 77-12, Ranjan, Subrita, VL 1, VL 4, VL 103	L 4147	IPL 81, LL 56

Response to GA_3 for root elongation		
Non responsive	**Responsive to 20 ppm**	**Responsive to 30 ppm**
Asha, DPL 15, JL 1, K 75, L 4076, L 4147, LH 84-6, LL 147, PL 406, PL 5, PL 639, PL 77-12, Ranjan, Subrita, VL 4, VL 1, VL 103	DPL 62, IPL 81, JL 3, LL 56, LL 699, NDL 1, PL 4	PL 234

639); and light brown (VL 103). Varieties that turn brown on reaction with phenol are categorized on the basis of response to GA_3, for shoot elongation as non-responsive (PL 406, PL 639) and responsive at 20 ppm (L 4147). Brown seeded varieties of small size are further classified on the basis of reaction with KOH and bleach, as brown (PL 4) and dark brown (LL 56, LL 147, LL 699). Varieties that turn brown on reaction with KOH-bleach are further classified on the basis of response to GA_3, for shoot elongation as non-responsive (LL 147, LL 699) and responsive at 30 ppm (LL 56). Black seeded varieties of small size are differentiated by testa mottling as presence of mottling (VL 4) and absence of mottling (VL 1). Medium seeded varieties are further categorized on the basis of seed coat colour as, grey (DPL 15, IPL 81, JL 1, K 75, L 4076, LH 84-6, NDL 1, PL 77-12, PL 234); and pink (JL 3, Subrita). Grey, medium sized varieties are distinguished on the basis of activity as very light brown (JL 4); light brown (DPL 15, IPL 81, L 4076, LH 84-6, NDL 1, PL 77-12, PL 234); and brown (K 75). Varieties with medium hydrogen-peroxidase activity are further categorized on the basis of phenol colour test as black (DPL 15, IPL 81, PL 77-12, L 4076, NDL 1); and brown (LH 84-6,

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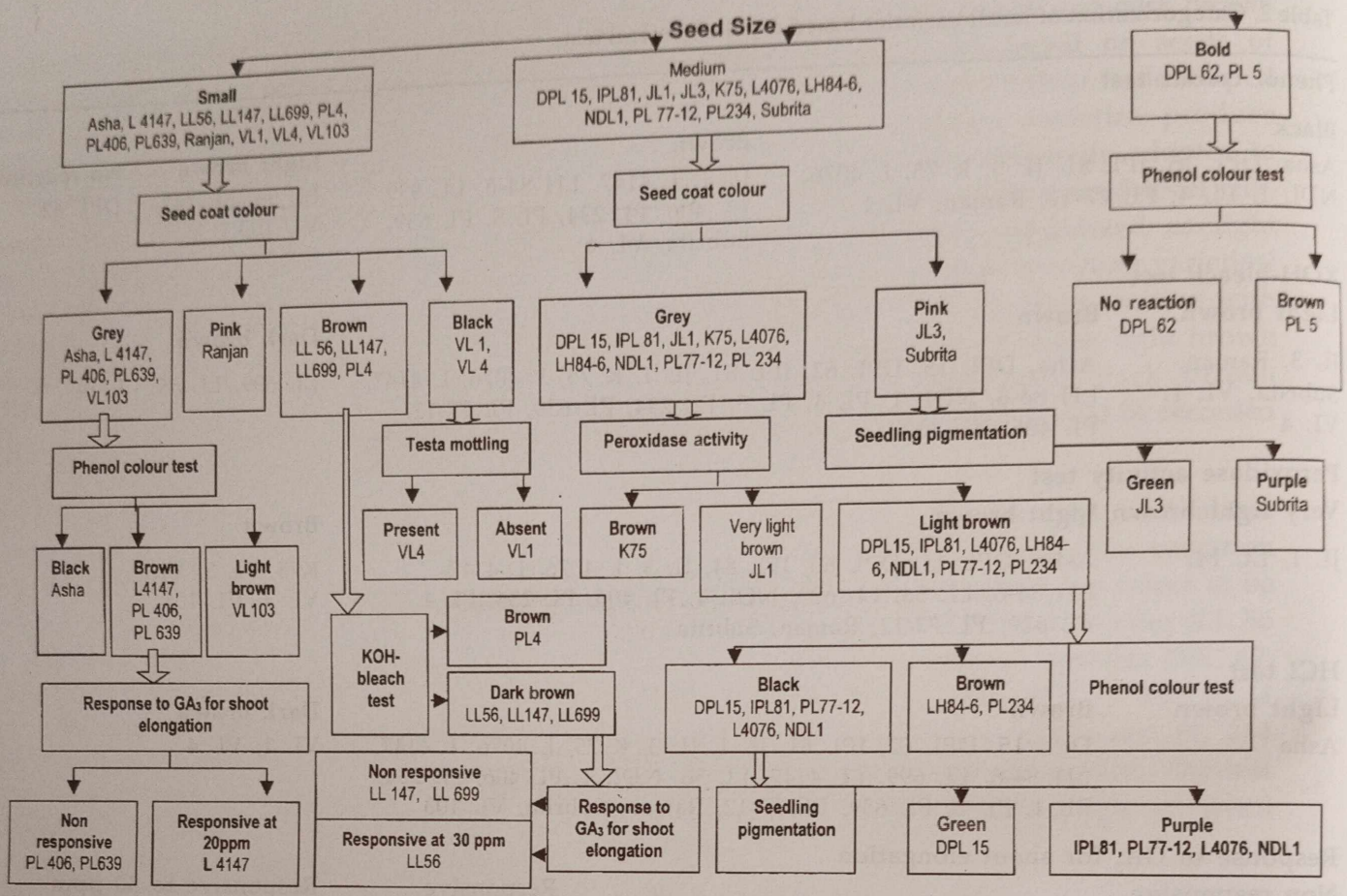

Fig. 1. Key for verification of lentil varieties at seed level

Table 3. Uniformity for the expression of the observed traits on seed of lentil based on off-type percentage

Character	Expression of off-type	Off-type (%)	Variety
Seed coat colour	-	-	-
Cotyledon colour	Yellow cotyledon	10	PL 5
Testa mottling	-	-	-
Seedling pigmentation	Green epicotyl	7	NDL 1
Phenol colour test	Non responsive	6	PL 5
KOH-bleach test	-	-	-
Peroxidase activity test	-	-	-
KOH test	-	-	-
HCI test	-	-	-
Response to GA_3	-	-	-

PL 234). Varieties that turn black on reaction with phenol are distinguished on the basis of seedling pigmentation as green (DPL 15) and purple (IPL 81, PL 77-12, L 4076, NDL 1). However varieties with purple epicotyl may not be further distinguished at seed level. Pink varieties of medium size may be distinguished on the basis of seedling pigmentation as green (JL 3) and purple (Subrita). Bold seeded varieties may be distinguished on the basis of reaction with phenol as non-reactive (DPL 62) and brown (PL 5).

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