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Full Length Research Paper

# Morphological characterization in onion (*Allium cepa* L.) for preparation and implementation of plant variety protection (PVP) legislation and distinctness, uniformity and stability (DUS) testing under temperate conditions of Kashmir

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The objective of this study was to analyze the variation in morphological characteristics of 30 varieties of onion for use as genetic markers. Identification keys for characterizing 30 onion varieties were employed on plant morphology and marked differences were observed among the varieties so that the new onion varieties could be identified, released and patented on the basis of the key. Morphological traits are thus widely accepted in plant variety protection, registration, and inscription and patenting as descriptors that are capable of showing both identity and distinctness.

**Key words:** Cultivar identification, onion, morphology, plant variety protection, distinctness, uniformity, stability (DUS) testing.

# INTRODUCTION

Government of India has enacted legislation on Protection of Plant Varieties and Farmers Rights (PPV & FR) in 2001 to provide legal framework for Plant Breeders' and Farmers Rights. The rules of this Act have been notified in September, 2003. Plant variety authority in India came into existence under this Act, which is responsible for implementation of this Act. Plant varieties seeking protection need to be registered with plant variety protection (PVP) authority. For registration, the varieties have to pass through distinctiveness, uniformity and stability (DUS) test of characters over generations. Descriptions thus define proprietary products of plant breeding and allow inscription or protection of novel varieties through PVP or utility patents. Accurate morphological descriptions of cultivars have proved reliable and provided the basis of assurance to farmers and merchants that they are being offered specific varieties or classes of product to certain minimum standards of quality and purity. Though many tools are now available to study relationship among cultivars

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including various types of molecular markers, however, morphological characterization is the first step in description and classification. It is commonly known that morphological data can be of dubious taxonomic reliability because of environmental interaction and the largely unknown mechanisms of genetic control of these traits (Comstock and Moll, 1963; Camussi et al., 1983). However, problems associated with the interpretation of morphological descriptions can be minimized by measuring traits in several environments or by limiting comparisons to those traits for which the effects of environmental interaction are smallest. Besides, continued usage of morphological features to describe varieties indicates that these morphological markers retain popularity as descriptors. It is likely that use of these descriptors will continue because they are omnipresent in agriculture (Smith and Smith, 1989a) and measurement and careful observations of morphological data would give an initial indication of any materials that were very similar (Smith and Smith, 1989b).

The intensive crop improvement programmes have resulted in diversification of varietal profile in the onion crop cultivated in India and one of the major thrusts at present is to maintain their purity besides registration (patents) and commercial release of new verities. Therefore, an attempt was made to characterize most of the already released onion varieties over years so as to develop identification keys that are reliable and relatively easy to perform while implementing PVP legislation and DUS testing in onion under temperate conditions of Kashmir

#### MATERIALS AND METHODS

The present investigation was carried out for successive 3 years during 2004 to 2005, 2005 to 2006 and 2007 to 2008 to carry out characterization of already released onion varieties (30) at Experimental Field of Division of Olericulture, SKUAST-K, Shalimar, Srinagar, India. The experimental material comprised 30 varieties (Pusa White Round, VL-3, Pusa Madhavi, N-2-4-1, Arka Pragati, Pillpatti Local, Pusa Red, Udaipur -102, Phule Suvarna, PRO-6, Hisar-2, Arka Niketan, Panjab Naroya, B-4, Agrifound Rose, Gujarat White Onion, Agrifound Light Red, Arka Pitambar, Yellow Globe, Agrifound Dark Red, B-780(V), B-780(MPKV), Arka Kalyan, N-53(MPKV), Phule Samartha, B-780, N-53, Phule Safed, Fursungi Local and Taloja Red), sown in a bed (1.8 × 2.0 m<sup>2</sup>) with row-to-row and plant-to-plant spacing of 15 ×10 cm, in randomized block design with three replications. In each replicate, all recommended package of practices were followed to raise a good crop (Table 1).

#### Statistical analysis

The mean value of 10 randomly selected plants in each plot over years was used for statistical analysis in traits where numerical values were taken, as suggested by Panse and Sukhatme (1978).

## **RESULTS AND DISCUSSION**

The morphological markers in 30 varieties of onion are

presented in Table 2. In the present study, there was no variation among onion varieties for few traits viz., leaf glossiness (1-absent), bulb bolting tendency except Phule Samartha (5-bolting), bulb cross section (1-assymetrical) and seasonal adaptability (1-*rabi*). Thus, onion varieties are very difficult to be differentiated on the basis of these traits. However, varieties evaluated for rest of the traits differed clearly from each other and form very reliable morphological descriptor profile. The descriptors have been explained separately on the basis of foliage and bulb characteristics.

## **Foliage descriptors**

Foliage attitude of N-2-4-1, Pillpati Local, Pusa Red, Udaipur-102, Arka Niketan, B-4, Agrifound Rose, Agrifound Light Red and Arka Kalyan was erect (1); Pusa White Round, VL-3, Pusa Madhavi, Arka Pragati, Phule Suvarna, Hissar-2, Gujarat White Onion, Arka Pitamber, Yellow Globe, Agrifound Dark Red, B-780 (V), B-780 (MPKV), N-53 (MPKV), Phule Samartha, B-780,N-53, Phule Safed, Fursungi Local and Taloja Red was semi erect (2), while horizontal (3) in PRO-6 and Punjab Naroya. Leaf waxiness was strong (7) in N-53 (MPKV), B-780, N-53, Fursungi Local and Taloja Red; medium (5) in Agrifound Dark Red only, while rest of the varieties evaluated exhibited weak (3) leaf waxiness. Leaf colour was light green (1) in PRO-6, Arka Niketan, B-4, Gujarat White Onion, Agrifound Light Red, Agrifound Dark Red, Arka Kalyan, N-53(MPKV), Phule Samartha, B-780, N-53 and Phula Safed; medium green in Pusa White Round, Arka Pragati, Pillpati Local, Pusa Red, Hissar-2, Punjab Naroya, Agrifound Rose, B-780 (V), B-780 (MPKV) and Fursungi Loca, I while dark green (7) in rest of the varieties. Leaf foliage fall was high (7) in B-780 (V), Arka Kalyan, Phule Samartha and N-53; medium (5) for B-780 (MPKV) and B-780; weak (3) for Agrifound Dark Red and N-53 (MPKV) while very weak (1) in rest of the varieties. Foliage cranking was absent/very weak (1) in Pusa White Round, Arka Pragati, Hissar-2, Arka Niketan, B-4, Agrifound Light Red and Taloia Red: weak (3) in VL-3. N-2-4-1, Pillpati Local, Udaipur-102, Gujarat White Onion, Arka Pitamber, Agrifound Dark Red, B-780 (V), Arka Kalyan, Phule Samartha, B-780, Phule Safed and Fursungi Local; medium (5) in Pusa Madhavi, Pusa Red, Phule Suvarna, PRO-6, Punjab Naroya, Agrifound Rose, Yellow Globe and N-53, while strong (7) in rest of the varieties studied (Figure 1).

## **Bulb descriptors**

Bulb shape was flat/thick flat (9) in VL-3, N-2-4-1, Pusa Red, Hissar-2, Agrifound Rose, Agrifound Light Red, Fursungi Local and Taloja Red, while flat globe/globe (6) in rest of the varieties studied. Bulb thickness of skin (mm) was thin (3) in Pusa White Round, VL-3, N-2-4-1, Arka Pragati, Udaipur-102, Phule Suvarna, PRO-6, Arka

S/N	Characteristics	States	States	States	States
1	Foliage attitude	Erect-1	Semi erect-2	Horizontal-3	
2	Leaf waxiness	Absent-1	Weak-3	Medium-5	Strong-(7)
3	Leaf colour	light green-1	Medium green-2	Dark green-3	Bluish(9)
4	Leaf glossiness	Absent-1	Weak-3	Medium-5	Strong(7)
5	Foliage fall	Absent -1 (<11%)	Weak-3 (11 - 25%)	Medium-5 (26 - 50%)	High-(7) >50%
6	Foliage cranking	Absent-1	Weak-3, Very strong-9	Medium-5, Strong-7	
7	Bulb shape	Flat globe/globe-6	Thick flat/flat-9		
8	Bulb thickness of skin	Thin-3 (<0.05 mm)	Medium-5 (0.05 - 0.06 mm)	Thick-7 (>0.07 mm)	
9	Bulb colour of skin	White-1, Yellow-2	Dark yellow-3, Bronze-4, Orange brown-5	Pale orange red-6 Pale red-7	Reddish brown-8 Dark red-9
10	Bulb colour of flesh	Weak-1	Reddish-2	Purplish-(3),	
11	Bulb skin adherence	Weak-3	Medium-5	Strong-(7)	
12	Bulb position of root disc	Deep-3	Flat-5	Outstanding(7)	
13	Bulb predominant number of axes	One-1	Two-2	Three-3	
14	Bulb cross section	Assymetrical-1	Symmetrical-2		
15	Bulb bolting tendency	Absent/very weak-1 (<10%)	Weak-3 (11 - 25%)	Medium-5 (26 - 50%)	Strong-7 (>50%)
16	Bulb degree of splitting bulblets	Absent-1	Weak-3 (<11%)	Medium-5 (11 - 20%)	High-7 (>21 - 30%)
17	Seasonal adaptability	Rab-1	kharief-2	late kharief-3	

Table 1. Descriptions of analysis as per Lawande and Krishna (2003).

Niketan, Punjab Naroya, B-4, Agrifound Rose, Agrifound Light Red, Arka Pitamber, Fursungi Local and Taloja Red; medium (5) in Pusa Madhavi, Pillpati Local, Pusa Red, Hissar-2, Gujarat White Onion, Yellow Globe and Phule Safed while thick (7) in rest of the varieties. Bulb colour of skin was white (1) in Pusa White Round, Udaipur-102, B-4, Gujarat White Onion and Phule Safed, yellow (2) in Yellow Globe; red (6-9) in rest of the varieties ranging from pale red in VL-3, Pusa Madhavi, Arka Pragati, Pillpati Local, Pusa Red, Hissar-2, Arka Niketan ,Punjab Naroya , Agrifound Light Red, Arka Pitamber; dark red in PRO-6 and Agrifound Rose and red in remaining varieties. Onion bulb colour is controlled at least by five major loci (I,C,G,L and R) (Anil, 2008) and stress is laid on bulb pigments as it is related to occurrence of protocatechuic acid which has medicinal effects besides conferring disease resistance in onion (Link et al., 1929). Bulb colour of flesh was purplish (3) in Pillpati Local, B-4, Agrifound Dark Red, B-780 (MPKV), Arka Kalyan, Phule Samartha, B-780 and Taloja Red; reddish (2) in Phule Suvarna, N-53 (MPKV) and N-53, while white (1) in rest of the varieties evaluated except vellowish white in Yellow Globe. Bulb skin adherence was weak (3) in Pusa White Round, VL-3, Pusa Madhavi, Arka Pragati, Udaipur-102, B-4, Agrifound Rose, Gujarat White Onion, Agrifound Light Red, Agrifound Dark Red, B-780 (V), N-53 (MPKV), Phule Samartha, N-53 and Taloja Red; medium (5) in N-2-4-1, Pillpati Local, Pusa Red, Phule Suvarna, PRO-6, Hissar-2, Arka Niketan, Punjab Naroya, Arka Pitamber, Yellow Globe, B-780 (MPKV), Arka Kalyan, B-780, Phule Safed, Fursungi Local, while none of the varieties exhibited strong (7) skin adherence. Bulb position of root disc was deep (3) in Agrifound Rose, B-780 (V), B-780 (MPKV), outstanding (7) in Agrifound Dark Red and N-53, while flat (5) in rest of the varieties evaluated. Bulb predominant number of axes varied considerably within each variety (Figure 2). However, mean values obtained are presented against each variety in Table 2.

The genotypes studied under temperate conditions of the valley remained satisfactory for most of the traits. These genotypes shall be subjected to massing and selfing for further improvement or involved in hybridization programme for development of hybrids.

The trait has been found to be very difficult to be considered for DUS testing so far its expression under temperate conditions of Kashmir is concerned. Bulb splitting note bulblets was very high (9) in Agrifound Dark Red, medium (5) in PRO-6, B-780 (V), B-780 (MPKV), Taloja Red; low (3) in Arka Kalyan, N-53 (MPKV), Phule Samartha, B-780, N-53, Phule Safed and Fursungi Local, while absent (1) in rest of the varieties.

On the basis of above investigation, it can be concluded that onion varieties can be easily differentiated from one another due to their distinctive, uniform and stable expression of morphological markers over years, thus, lending support to the earlier evidences that the morphological characterization continues to be an effective and reliable tool in DUS testing.

## ACKNOWLEDGEMENT

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Figure 1. Photographs of onion varieties evaluated under DUS.



Figure 2. Photographs of onion varieties evaluated under DUS.

Table 2. Morphological markers recorded in different onion (Allium cepa L.) varieties under temperate conditions of Kashmir.

C/N	Name of	Foliage	Leaf	Leaf	Leaf	Leaf foliage	Foliage/leaf	Bulb	Bulb thickness	Bulb colour of
3/N	variety	attitude	waxiness	colour	glossiness	fall (%)	cranking	shape	of skin (mm)	skin
1	Pusa White Round	Semi erect(2)	Weak(3)	Medium green(2)	Absent(1)	Absent(1)	Ab./very weak(1)	Flat globe(6)	0.03(3)	White(1)
2	VL-3	Semi erect(2)	Weak(3)	Dark green(3)	Absent(1)	Absent(1)	Weak(3)	Flat(9)	0.03(3)	Pale red(7)
3	Pusa Madhavi	Semi erect(2)	Weak(3)	Dark green(3)	Absent(1)	Absent(1)	Medium(5)	Flat globe(6)	0.05(5)	Pale red(7)
4	N-2-4-1	Erect(1)	Weak(3)	Dark green(3)	Absent(1)	Absent(1)	Weak(3)	Flat(9)	0.03(3)	Pale orange red(6)
5	Arka Pragati	Semi erect(2)	Weak(3)	Medium green(2)	Absent(1)	Absent(1)	Ab./very weak(1)	Flat globe(6)	0.03(3)	Pale red(7)
6	Pillpatti Local	Erect(1)	Weak(3)	Medium green(2)	Absent(1)	Absent(1)	Weak(3)	Flat globe(6)	0.04(5)	Pale red(7)
7	Pusa Red	Erect(1)	Weak(3)	Medium green(2)	Absent(1)	Absent(1)	Medium(5)	Thick flat(9)	0.05(5)	Pale red(7)
8	Udaipur -102	Erect(1)	Weak(3)	Dark green(3)	Absent(1)	Absent(1)	Weak(3)	Flat globe(6)	0.02(3)	White(1)
9	Phule Suvarna	Semi erect(2)	Weak(3)	Dark green(3)	Absent(1)	Absent(1)	Medium(5)	Globe(6)	0.03(3)	Yellow(2)
10	PRO-6	Horizontal(3)	Weak(3)	Light green(1)	Absent(1)	Absent(1)	Medium(5)	Flat globe(6)	0.02(3)	Dark red(9)
11	Hisar-2	Semi erect(2)	Weak(3)	Medium green(2)	Absent(1)	Absent(1)	Ab./very weak(1)	Flat(9)	0.06(5)	Pale red(7)
12	Arka Niketan	Erect(1)	Weak(3)	Light green(1)	Absent(1)	Absent(1)	Ab./very weak(1)	Globe(6)	0.03(3)	Pale red(7)
13	Panjab Naroya	Horizontal(3)	Weak(3)	Medium green(2)	Absent(1)	Absent(1)	Medium(5)	Flat globe(6)	0.03(3)	Pale red(7)
14	B-4	Erect(1)	Weak(3)	Light green(1)	Absent(1)	Absent(1)	Ab./very weak(1)	Flat globe(6)	0.02(3)	White(1)
15	Agrifound Rose	Erect(1)	Weak(3)	Medium green(2)	Absent(1)	Absent(1)	Medium(5)	Flat(9)	0.03(3)	Dark red(9)
16	Gujarat White Onion	Semi erect(2)	Weak(3)	Light green(1)	Absent(1)	Absent(1)	Weak(3)	Globe(6)	0.04(5)	White(1)
17	Agrifound Light Red	Erect(1)	Weak(3)	Light green(1)	Absent(1)	Absent(1)	Ab./very weak(1)	Flat(9)	0.01(3)	Pale red(7)
18	Arka Pitambar	Semi erect(2)	Weak(3)	Dark green(3)	Absent(1)	Absent(1)	Weak(3)	Globe(6)	0.01(3)	Pale red(7)
19	Yellow Globe	Semi erect(2)	Weak(3)	Dark green(3)	Absent(1)	Absent(1)	Medium(5)	Flat globe(6)	0.06 (5)	Yellow(2)
20	Agrifound Dark Red	Semi erect(2)	Medium (5)	Light green(1)	Absent(1)	Weak (3)	Weak(3)	Flat globe(6)	0.08 (7)	Dark red(9)
21	B-780(V)	Semi erect(2)	Weak(3)	Medium green(2)	Absent(1)	High (7)	Weak(3)	Flat globe(6)	0.09 (7)	Dark red(9)
22	B-780(MPKV)	Semi erect(2)	Weak(3)	Medium green(2)	Absent(1)	Medium (5)	Strong (7)	Flat globe(6)	0.10 (7)	Pale Red (7)
23	Arka Kalyan	Erect(1)	Weak(3)	Light green(1)	Absent(1)	High (7)	Weak(3)	Flat globe(6)	0.09 (7)	Dark red(9)
24	N-53(MPKV)	Semi erect(2)	Strong (7)	Light green(1)	Absent(1)	Weak (3)	Strong (7)	Flat globe(6)	0.09 (7)	Dark red(9)
25	Phule Samartha	Semi erect(2)	Weak(3)	Light green(1)	Absent(1)	High (7)	Weak(3)	Globe(6)	0.09 (7)	Dark red(9)
26	B-780	Semi erect(2)	Strong (7)	Light green(1)	Absent(1)	Medium (5)	Weak(3)	Flat globe(6)	0.07 (7)	Dark red(9)
27	N-53	Semi erect(2)	Strong (7)	Light green(1)	Absent(1)	High (7)	Medium(5)	Flat globe(6)	0.08 (7)	Dark red(9)
28	Phule Safed	Semi erect(2)	Weak (3)	Light green(1)	Absent(1)	Absent(1)	Weak(3)	Globe(6)	0.07 (5)	White (1)
29	Fursungi Local	Semi erect(2)	Strong (7)	Medium green(2)	Absent(1)	Absent(1)	Weak(3)	Flat(9)	0.05 (3)	Dark red(9)
30	Taloja Red	Semi erect(2)	Strong (7)	Dark green(3)	Absent(1)	Absent(1)	Ab./very weak(1)	Flat(9)	0.06 (3)	Dark red(9)
S/N	Name of variety	Bulb colour of flesh	Bulb skin adherence	Bulb colour of flesh	Bulb position of root disc	Bulb predominant number of axes	Bulb cross section	Bulb bolting tendency (%)	Splitting note bulb lets (%)	Seasonal adaptability
1	Pusa White Round	White(1)	Weak (3)	White(1)	Flat(5)	3.83(4)	1.46(Asymmetrical)	Absent(1)	Absent(1)	Rabi(1)

Table 2. Contd.

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2	VL-3	White(1)	VVeak (3)	vvnite(1)	Flat(5)	2.60(3)	1.40(Asymmetrical)	Absent(1)	Absent(1)	Rabi(1)
3	Pusa Madhavi	White(1)	Weak (3)	White(1)	Flat(5)	3.93(4)	1.66(Asymmetrical)	Absent(1)	Absent(1)	Rabi(1)
4	N-2-4-1	White(1)	Medium(5)	White(1)	Flat(5)	1.86(2)	1.36(Asymmetrical)	Absent(1)	Absent(1)	Rabi(1)
5	Arka Pragati	White(1)	Weak (3)	White(1)	Flat(5)	3.90(4)	1.60(Asymmetrical)	Absent(1)	Absent(1)	Rabi(1)
6	Pillpatti Local	Purplish(3)	Medium(5)	Purplish(3)	Flat(5)	2.63(3)	1.60(Asymmetrical)	Absent(1)	Weak(3)	Rabi(1)
7	Pusa Red	White(1)	Medium(5)	White(1)	Flat(5)	3.00(3)	1.70(Asymmetrical)	Absent(1)	Absent(1)	Rabi(1)
8	Udaipur -102	White(1)	Weak (3)	White(1)	Flat(5)	4.13((4)	1.85(Asymmetrical)	Absent(1)	Absent(1)	Rabi(1)
9	Phule Suvarna	Reddish(2)	Medium(5)	Reddish(2)	Flat(5)	3.9(4)	1.53(Asymmetrical)	Absent(1)	Absent(1)	Rabi(1)
10	PRO-6	White(1)	Medium(5)	White(1)	Flat(5)	2.66(3)	1.40(Asymmetrical)	Absent(1)	Medium (4)	Rabi(1)
11	Hisar-2	White(1)	Medium(5)	White(1)	Flat(5)	2.93(3)	1.40(Asymmetrical)	Absent(1)	Absent(1)	Rabi(1)
12	Arka Niketan	White(1)	Medium(5)	White(1)	Flat(5)	2.86(3)	1.33(Asymmetrical)	Absent(1)	Absent(1)	Rabi(1)
13	Panjab Naroya	White(1)	Medium(5)	White(1)	Flat(5)	2.5(3)	1.56(Asymmetrical)	Absent(1)	Absent(1)	Rabi(1)
14	B-4	Purplish(3)	Weak (3)	Purplish(3)	Flat(5)	3.06(4)	1.40(Asymmetrical)	Absent(1)	Absent(1)	Rabi(1)
15	Agrifound Rose	White(1)	Weak (3)	White(1)	Deep(3)	3.30(4)	1.56(Asymmetrical)	Absent(1)	Absent(1)	Rabi(1)
16	Gujarat White Onion	White(1)	Weak (3)	White(1)	Flat(5)	2.06(2)	1.50(Asymmetrical)	Absent(1)	Absent(1)	Rabi(1)
17	Agrifound Light Red	White(1)	Weak (3)	White(1)	Flat(5)	1.90(2)	1.10(Asymmetrical)	Absent(1)	Absent(1)	Rabi(1)
18	Arka Pitambar	White(1)	Medium(5)	White(1)	Flat(5)	2.13(2)	1.43(Asymmetrical)	Absent(1)	Absent(1)	Rabi(1)
19	Yellow Globe	White(1)	Medium(5)	White(1)	Flat(5)	2.36(2)	1.33(Asymmetrical)	Absent(1)	Absent(1)	Rabi(1)
20	Agrifound Dark Red	Purplish(3)	Weak (3)	Purplish(3)	Outstanding (7)	3.40 (4)	1.35 Asymmetrical	Absent(1)	Very high (9)	Rabi(1)
21	B-780(V)	White(1)	Weak (3)	White(1)	Deep(3)	3.33 (4)	1.36(Asymmetrical)	Absent(1)	Medium (5)	Rabi(1)
22	B-780(MPKV)	Purplish(3)	Medium(5)	Purplish(3)	Deep(3)	2.90(2)	1.30(Asymmetrical)	Absent(1)	Medium (5)	Rabi(1)
23	Arka Kalyan	Purplish(3)	Medium(5)	Purplish(3)	Flat(5)	3.63 (4)	1.46(Asymmetrical)	Absent(1)	Low (3)	Rabi(1)
24	N-53(MPKV)	Reddish (2)	Weak (3)	Reddish (2)	Flat(5)	3.27 (4)	1.56(Asymmetrical)	Absent(1)	Low (3)	Rabi(1)
25	Phule Samartha	Purplish(3)	Weak (3)	Purplish(3)	Flat(5)	3.03 (4)	1.20(Asymmetrical)	Bolting (5)	Low (3)	Rabi(1)
26	B-780	Purplish(3)	Medium(5)	Purplish(3)	Flat(5)	3.03 (4)	1.25(Asymmetrical)	Absent(1)	Low (3)	Rabi(1)
27	N-53	Reddish (2)	Weak (3)	Reddish (2)	Outstanding (7)	2.97(2)	1.33(Asymmetrical)	Absent(1)	Low (3)	Rabi(1)
28	Phule Safed	White(1)	Medium(5)	White(1)	Flat(5)	3.10 (4)	1.20(Asymmetrical)	Absent(1)	Low (3)	Rabi(1)
29	Fursungi Local	White(1)	Medium(5)	White(1)	Flat(5)	2.70(2)	1.30(Asymmetrical)	Absent(1)	Low (3)	Rabi(1)
30	Taloja Red	Purplish(3)	Weak (3)	Purplish(3)	Flat(5)	3.40 (4)	1.43(Asymmetrical)	Absent(1)	Medium (5)	Rabi(1)

Varieties from S/N 1 to 19 were evaluated during 2004 to 2005, 2005 to 2006 and 2006 to 2007, while from S/N 20 to 30 were evaluated during 2005 to 2006,2006 to 2007 and 2007 to 2008 (3 years).

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