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Short Communication

RAPD Markers and Morphological Characteristics for Identification of French bean (*Phaseolus vulgaris* L.) Cultivars

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French bean (*Phaseolus vulgaris* L.) is one of the important vegetable pulses grown extensively in the tropical and sub-tropical areas of the world. In India, it is consumed both as green vegetable as well as dry seeds. This is a rich source of carbohydrates (61.4%), proteins (17.5 to 28.5%) minerals (3.2 to 5.0%) and vitamins [1]. Under the plant variety protection regime a new variety can be protected only if it is distinct from other varieties, uniform in their characteristics and genetically stable. Description of characters for the assessment of varietal purity and identity are also essential in seed production and seed certification programmes [2]. However, Grow Out Test (GOT) is a time consuming process and some time require skilled labour and to be conducted in off-season. Hence, there is a need to develop rapid techniques at molecular level. Electrophoretic techniques at protein and DNA level is one of the advanced techniques used to identify the given variety in the seed production and certification programme [3]. Hence, an attempt has been made to study the morphological characteristics and DNA marker technique for identification of french bean varieties.

Genetically pure and fresh seeds of seven different french bean varieties viz., RSJ-288, IIHR-9-9 (Arka Suvidha), MFB-1, Contender, Arka Komal MFB-2 and MFB-3 were collected from the AICRP on Vegetables, UAS, Dharwad to carry out studies pertaining to identification of french bean varieties based on seed, seedling plant and RAPD analysis using PCR. The various seed morphological parameter such as seed coat color, hilum color, seed shape were recorded. Fifty seeds were sown in sand media of which five day old ten normal seedlings were taken for recording seedling morphological characters viz., hypocotyl color, cotyledon color, and pubescence on hypocotyls. The color of flower from each variety grown separately, two lines of five meter length at flowering stage are observed and grouped as dark purple, pale purple and white. Similarly constriction on pod was also observed under field condition and categorized as Shallow, Moderate and Deeply Constricted.

Fresh young leaf sample from shoot apex were collected from selected seedling and DNA was isolated using slightly modified CTAB maxi-prep method [4]. The DNA was quantified on 0.7-0.8 per cent Agarose gel and diluted to serial dilutions which was carried out to get desired quantity of DNA for PCR. Following primers from Operon Technologies, USA were used in PCR.

Code	5 TO3′
OPF-07	CCGATATCCC
OPF-10	GGAAGCTTGG
OPF-16	GGAGTATGG
OPF-20	GGTCTAGAGG

After completion of the reaction, amplified DNA was separated using 1.2 per cent Agarose gel in 1 X TAE and 0.5 mg/µl Ethidium. The contents were electrophoresed and visualized with UV documentation system (Herolab).

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In the present investigation seven french bean varieties were grouped into seven seed coat color i.e. black, pale brown, brown, light brown, mottled, creamy white and dark brown (Table 1). Though seed coat color is a heritable character it is also influenced by environmental conditions during ripening [5]. The hilum color of seed was found to be white in all the varieties [1]. Seed shape for different french bean varieties were grouped into Cylindrical (Contender, Arka Komal) and Kidney shaped (IIHR-909). Whereas, MFB-1, MFB-2, MFB-3 are grouped as oval shape. The variety RS-288 could be identified individually from other variety. This may be due to the position of seed in the pod and may be influenced by environmental conditions during the pod filling stage.

The seedling morphological character such as pubescence on hypocotyls varieties are categorized into Dence (hairy) and Glabrous (non-hairy). The varieties like RSJ-288, IIHR-909, Arka Komal are grouped into dense hairy group, whereas MFB-1. Contender and MFB-2 are grouped under Glabrous. This variation is due to genetic characters of varieties as reported by Pathak and Singh [6]. Similar results were also reported in soybean [7]. Based on cotyledon color french bean varieties are grouped into purple, green and light green. RS-288, has purple cotyledon, whereas IIHR-909, MFB-3, MFB-2, MFB-1 and Arka Komal are categorized under green. The Contender is placed under light green group. The hypocotyl color of seedling varied with varieties viz., RSJ-288 (purple), IIHR-909 (green), Arka Komal (yellowish green), MFB-3 (light purple), MFB-1 and MFB-2 (pale green) and Contender (light green). This character is under genetic control [8]. These observations are in accordance with as reported in Blackgram [9].

The french bean varieties based on flower color as RSJ-288 (dark purple), Contender and MFB-2 (purple), MFB-3 (light purple), Arka Komal and IIHR-909 (purplish white) and MFB-1 (white). The variation in flower among the varieties is due to genotypic character. Similar work was carried out by various workers [7]. Based on pod constriction the varieties RSJ-288 and MFB-2 are grouped under shallow, Contender, MFB-1 and IIHR-909 as moderate and Arka Komal under deep constriction, while MFB-3 is having very deep pod constriction.

RAPD Analysis

RAPD analysis has a discriminatory potential as it probes the nucleotide composition of a gene rather than its products. Another advantage of this

Table 1. Seed, seedling and plant morphological characters of french bean varieties

S.No.	Varieties	Seed coat color	Seed hilum color	Seed shape	Hypocotyl color	Seedling cotyledon color	Pubescence on hypocotyls	Flower color	Constriction on pod
1.	RSJ-288	Black	White	Cylinder	Purple	Purple	Dense	Dark purple	Shallow
2.	IIHR-909	Light brown	White	Kidney	Green	Green	Dense	Purplish white	Moderate
3.	MFB-1	Creamy white	White	Oval	Pale green	Green	Glabrous	White	Moderate
4.	Contender	Pale brown	White	Kidney	Light brown	Light green	Glabrous	Purple	Deep
5.	Arka Komal	Brown	White	Kidney	Yellowish green	Green	Dense	Purplish white	Deep
6.	MFB-2	Mottled	White	Oval	Pale green	Green	Glabrous	Purple	Shallow
7.	MFB-3	Dark brown	White	Oval	Light purple	Green	Dense	Light brown	Very deep

method lies in the availability of innumerable primers for screening purposes. RAPD s has been extensively used in identification of crop varieties [10]. In the present study seven french bean varieties were analysed using RAPD technique. The primers of OPF series are screened. Among them, OPF-7, OPF-10, OPF-16 and OPF-20 exhibited polymorphic banding pattern. When OPF-7 primer is used. Contender and MFB-2 varieties exhibited an extra band of 4000 bp. These two varieties having a common seedling morphogenetic character like without pubescence (Glabrous) on hypocotyls. The varieties such as RSJ-288 and Contender are lacking a band of 600 bp, when primer OPF-10 is used for screening RSJ-288 is similar with other varieties like IIHR-909, Arka Komal and MFB-3 in morphological character like dense pubescence on hypocotyls and spready growth habit. By RAPD analysis RSJ-288 uniquely identified based on absence of 600 bp band when the primer OPF-16 used at distinctly identified the varieties namely MFB-1 and MFB-2 which are having an extra band of 1750 bp. When RAPD analysis is done using primer OPF-20, maximum varieties exhibited polymorphic banding pattern. Three varieties namely Contender, MFB-3 and MFB-1 can be distinctly identified using this primer though these varieties are similar for the character of glabrous hypocotyl no hairs on hypocotyls. The similar RAPD analysis method was employed by [11] to identify seven rice varieties using three primers, the banding pattern is key basis for identification of varieties. Such techniques were tried in tomato [12] and in common bean varieties [2]. Based on present discussion it is concluded that french bean varieties were identified with unique morphological character viz., variety RSJ-288 has black seed coat color, IIHR-909 produces dense hairiness on hypocotyl. The french bean could also be identified by using RAPD technique as a DNA marker *viz.*, variety Contender identified with polymorphic bands of 1400, 2200, and 300 bp using primer OPF-20 and MFB-2 identified with polymorphic bands of 170 bp using primer OPF-16.

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