Vol 5 Issue 5 June 2015

ISSN No : 2230-7850

International Multidisciplinary Research Journal

Indian Streams Research Journal

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RNI MAHMUL/2011/38595 **ISSN No.2230-7850**

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International Recognized Double-Blind Peer Reviewed Multidisciplinary Research Journal
Indian Streams Research Journal
ISSN 2230-7850 Impact Factor : 3.1560(UIF)
Volume - 5 | Issue - 5 | June - 2015 Available online at www.isrj.org

SEED PROTEIN VARIABILITY IN SIX DIFFERENT VARIETIES OF SAFFLOWER (*CARTHUMUS TINCTORIUS* L.) SEEDS BY SDS-PAGE.

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Short Profile

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ABSTRACT:
Total seed proteins in six different safflower (*Carthamus tinctorius* L.) variety Annigeri (A-1) (PURE), SSF-733, JLSF 414(Phulekusuma), PBNS-12 (Parbhanikusuma), SSE-707 and SF-11-16 have been separated by the SDS-PAGE method. Statistically significant differences in seed protein content were found among six safflower varieties. Results from these studies revealed that PBNS-12 variety is superior among other variety.

KEYWORDS
safflower, Protein, SDS-PAGE.

INTRODUCTION :

Safflower (*Carthamus tinctorius* L.) an oilseed crop is a member of the family Compositae or Asteraceae (Prakash, K.S. and B.G. Prakash. 1993). India is the largest producer of safflower in the world (350,000 ha), followed by Mexico (85000 ha), Ethiopia (72000 ha) and U.S (54000 ha). Safflower (*Carthamus tinctorius* L.) has become an oil crop used both for food and industrial purposes in many other countries (ZehraEkin, 2005). Safflower is an important oil seed crop of world and it ranks third in the production next to Groundnut and Soybean.

The aim of this study is to evaluate seed protein variability among the six different varieties.

MATERIALS AND METHODS:

Sample collection:

The safflower seeds were obtained from "krushi Kendra, solapur"(Maharashtra, india) include six different variety Annigeri (A-1)(PURE), SSF-733,JLSF 414(phulekusuma),PBNS-12 (parbhanikusuma),SSE-707 and SF-11-16 and were used for analysis.

Experimental procedure:

1.SDS-PAGE was carried out with the buffer system described by Laemmli (1970). Embryoless mature seeds (20 mg/0.5 ml) were suspended in a sample buffer of TRIS-HCl (pH 6.8), 2% SDS, 5% mercaptoethanol and 20% glycerol. After shaking for 2 h at room temperature, the suspension was boiled for 2 min. After centrifugation (5000 g) for 5 min at room temperature, 30 µl of protein solution were applied to a gel that consisted of 30.0% acrylamide and 0.8% bisacrylamide (10% C) along with broad range marker protein . Electrophoresis was carried out in vertical slab gels. gels were run at 100v for 2.5 hrs. Proteins were stained with 1% Coomassie Brilliant Blue R 250 in 40% methanol, 10% glacial acetic acid. Transfer the gel to a suitable container with at least 200-300 ml of destaining solution (40% methanol and 10% glacialacetic acid) and place in a shaker continuously. Dye that is not bound to proteins is thus removed. The destainer must be changed frequently, until the background of the gel is colourless. The protein bands are stained blue. The destaining process can be stopped at a stage, where many number of bands are visualised as much as possible. Faint bands represent minute quantities if proteins view at through a light box and score the bands for further analysis.

Calculate the Rf value of separated protein bands by using following formula.

Rf value =distance travelled by sample protein (cm)/ distance travelled by tracking dye (cm). (*Dragana R .Obreht et al 2002*).

RESULTS AND DISUSSION:

Total seed protein were separated by SDS-PAGE method. (Sodium dodecyl sulphate Polyacrylamide gel electrophoresis) (lamelli et al, 1970)

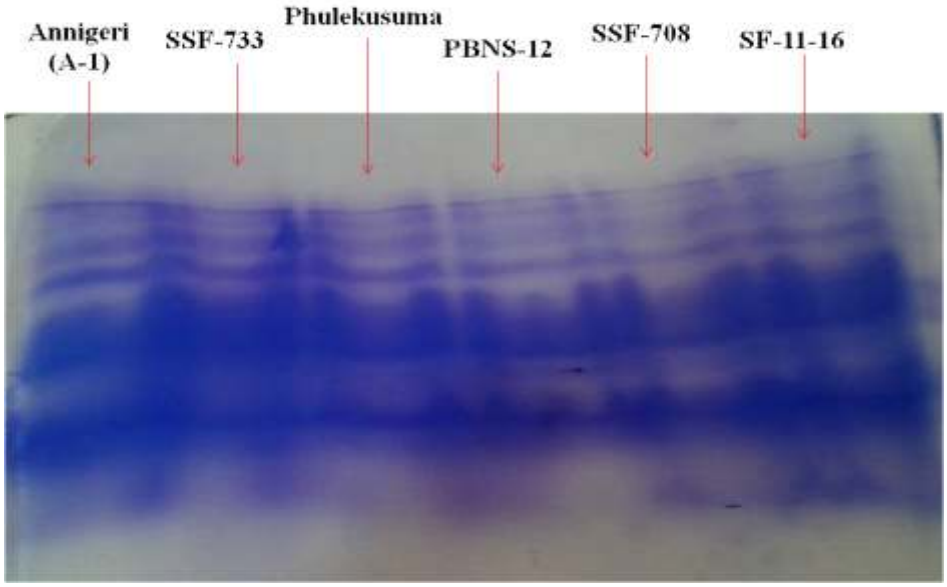

Fig. NO.1. SDS-PAGE of safflower variety seed protein.

Sr. No.	Name of safflower variety	No. Of bands.	Rf value of bands (Distance travelled by tracking dye = 6.5cm)							
			1st band	2nd band	3rd band	4th band	5th band	6th band	7th band	8th band
1.	Annigeri (A-1)	7	0.215	0.246	0.292	0.323	0.430	0.461	-	0.584
2.	SSF-733	7	0.261	0.292	0.323	0.375	0.461	0.492	-	0.615
3.	Phulekusuma	7	0.292	0.307	-	0.353	0.384	0.461	0.523	0.615
4.	PBNS-12	8	0.261	0.276	0.307	0.338	0.369	0.461	0.507	0.600
5.	SSF-708	8	0.261	0.276	0.307	0.369	0.446	0.507	0.523	0.615
6.	SF-11-16	7	0.230	0.276	-	0.307	0.369	0.461	0.492	0.584

Table No.1.Rf values of separated bands of safflower variety.

In the present work variation in proteins is observed by using SDS-PAGE. As per the observation six different varieties of safflower is analysed for protein profiling. In variety Annigeri (A-1) seven bands are observed, of these seven bands the Rf value was calculated as 0.215, 0.246, 0.292, 0.323, 0.430, 0.461 and 0.584. While as SSF-733 variety shows same seven number of bands but there Rf value is different i.e. 0.261, 0.292, 0.323, 0.375, 0.375, 0.461 and 0.615. The Rf value of 1st band, is same as that with the variety PBNS-12 and SSF-708.

In Phulekusuma variety also the number of bands is seven but the Rf values are different and

was found to be 0.292, 0.307, 0.353, 0.384, 0.461, 0.523 and 0.615. Compared to the work done by DraganaR.Obreth et.al., (2002) in two Safflower spp. (*Carthamus tinctorius* L. And *carthamus lanctus* L.). Their molecular masses ranged from 120 to 20 kDa. All *C. tinctorius* genotypes under study exhibited identical electrophoretic patterns which differed from the pattern exhibited by the wild species *C. Lanatus* in the number and position of protein bands. Differences in protein profiles occurred in regions around 60 kDa, from 43 to 36 kDa and around 30 kDa.

In safflower variety PBNS-12 the number of bands observed is 8 and the Rf calculated are 0.216,0.276, 0.307, 0.338, 0.369, 0.461, 0.507 and 0.600. The Rf value of 7th band is same as that of 7th band of variety SSF-708 and Phulekusuma.

Similarly in SSF-708 variety of safflower protein the bands observed are 8, while as the Rf values of first three bands are same as that of PBNS-12 variety, and the Rf value of other bands is 0.369, 0.446, 0.507, 0.523, and 0.615.

The SF-11-16 variety contains seven bands and there Rf values are found to be 0.230, 0.276, 0.307, 0.369, 0.461, 0.492 and 0.584.

CONCLUSION:

Protein electrophoresis is powerful tool for population genetics (parker et al 1998). As storage proteins are not affected by environmental changes their profiling using SDS-PAGE is considered as reliable method. All varieties show a great difference in their bands and Rf values. Hence these results can be used to study its genetic diversity.

The result of present investigation concludes that the protein profiling of different variety of safflower seeds shows variation by observing the bands on SDS-PAGE. In variety Annigeri (A-1), and there is difference in their Rf values. SSF-733, Phulekusuma and SF-11-16 the number of bands is seven, but there is difference in their Rf values. Also in variety PBNS-12 and SSF-708 the number of bands is same.

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