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Variation in Seed Protein Contains of Safflower by SDS- Page

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Abstract

The protein is an essential part of human diet. It plays an important role in human nutrition. So, the present study was conducted to investigate the variation in seed protein content in healthy and infected seeds of safflower. Two varieties of safflower i.e. var Sharda and var PBNS-12.was under taken to investigate the variation in seed protein content in healthy and seeds. sodium Dodecyl sulfate polyacrylamide Gel electrophoresis (SDS-PAGE) method was adopted. The band in I and II lane belongs to variety PBNS-12 healthy and diseased seeds. The bonds were compared with standard protein molecular weight marker in lane III. The protein content of var PBNS-12 both in healthy and disease seed ranges between 83.8 KD to 119 KD. Similar results obtained in variety Sharda in healthy and disease seeds. The molecular masses ranged from 26.2KD to 216KD. The significant difference in seed protein was recorded in seed protein content in healthy and infected seed of both varieties. In variety PBNS-12 the significant difference in seed protein was occurred in region 26.2 t 32. 7 KD and 199 KD to 216 KD. While rest of other regions shows similar banding patterns in both the varieties of healthy and infected seeds.

Keywords: *Safflower, seed protein, SDS-PAGE, Gel Electrophoresis, Molecular weight marker, Banding pattern*

Introduction

Safflower or Kardi (*Carthamus tinctorius L.*) an importance oil seed rabi crop from family. It contains 40 per cent edible oil which has high per cent of essential polysaturated fatty acids and linoleic acid which helps in reducing cholesterol level. It is used as colour in food processing industry. Significant difference in the content of total fatty oil, oleic and linoleic acids in safflower. Seeds collected in different countries and those collected from different sites in the same country (Kiss et al. 2001) (Dragna et al. 2002) studied total seed protein in two safflower species (Carthamus tinctorius L. and *Carthamus lanatus L.*). Jirair and Gholamerza (2005) conducted to find out the variation in protein oil and fatty acid content in 2 wild species in comparison to cultivated species. So, the present study was conducted to investigate the variation in seed protein content in healthy and infected seeds of safflower.

Material and Method:

These two varieties of safflower were collected from MAU, Parbhani. SDS-PAGE is a widely used method was developed by Laemmll (1970). The study was carried out on two safflower varieties were used var. PBNS-12 and Sharda.

Method: Seed protein Extraction method

Protein extraction method describer by Shewry (1995). The buffer is composed of several components, including Tris-Hcl, SDS, DTT, glycerol and MilliQ water.

Buffer composition for 10 ml (Final concentration)

1. 1M Tris-HCL, pH 6.8,: 0.625 ml resulting in a final concentration of 62.5 mM
2. 10% SDS(w/v):2.000ml,resulting in a final concentration of 2%.
3. 1MDTT: 1.000ml,resulting in a final concentration of 10mM
4. 100% Glycerol :1.000ml, resulting in a final concentration of 10%
5. MilliQ water: 5.375 ml

Protein Extraction Buffer (+DTT/+BB)

To prepare protein extraction buffer, combine 1ml of the buffer with a small amount of Bromophenol Blue (BB) powder on the tip of a wetted pipette tip in a microfuge tube. This buffer serves as a sample buffer for gel electrophoresis.

Method Used-

1. Seed sample preparation: Placed 5 seeds in each sample into 2 ml safe-lock microfuge tube.
2. Sample weight measurement: Weigh and record the weight of per sample.
3. Addition of Stainless steel Bead: Placed one 5 mm stainless steel bead to each tube.

Quantification of protein using the Bradford assay
Procedure:

Duplicate samples and standards are prepared for analysis. Pipette 800 µL Milli Q water to microfuge tube. Subsequently withdraw 1µL from it using a P2 Gilson pipette. as it's not feasible to pipette 799 µL using a P1000 Gilson pipette. Adjust the volume of water for other BSA standard based on the required amount of standard to be add at 1:1 ratio. For a 7µg standard, remove 7 µL of 1 µg/µL BSA standard. Add 200 µL of Bio-Rad protein Assay dye Reagent to it. Mix the solution briefly using a Vortex mixture. Allow the mixture to incubate at room temperature for at least 5 minutes. During this incubation period, turn on the spectrophotometer and adjust the wavelength to 595 nm. Once the incubation is complete, transfer the sample to disposable cuvette. Measure the absorbance of the sample at 595 nm and record the value. Enter the
standard curve and samples into Excel spreadsheet. Create an XY scatter plot with linear trendline to generate the standard curve. Finally use the Standard curve to determine concentration the of samples.

Separation of seed proteins by SDS-PAGE

Protein extracts (3 µg total protein per sample) are separated by SDS-PAGE according to the method of Laemmli (1970) on a Biorad Protean II system. The separation is performed using 15% resolving and 5% stacking gels, at 200 V for 55 min.

Tris-glycine gel buffer as per Sambrook 1989
Preparation: For 100 mL Tris-glycine gel buffer
The component and quantity are:
Trizma (1.51g,0.125M),Glycine (9.40g,0.125M) and 10%SDS(5.00ml,0.5%).The finally add MilliQ water to reach a total volume of 100 ml.

Gel preparation
1. Make the following gels in glass vials, without adding the 10% ammonium persulfate and TEMED.

The protocol then outlines the steps for assembling the gel using Biorad Protean II system, including the addition of 10% ammonium persulfate and TEMED to the resolving gel solution, pouring butan-1-ol across the top of the resolving gel, and allowing the gel to polymerize for approximately 45 minutes. After rinsing the butan-1-ol thoroughly with MilliQ water, the stacking gel solution is added, and the comb is placed into the stacking gel. Allow 45 min for gel to polymerise then removing comb and rinsing the wells with 1x Tris-glycine gel buffer to remove any unpolymerized gel.

Preparation of samples for SDS-PAGE
1. Standardize protein samples to 3µg in 10 µl using Protein Extraction Buffer containing DTT and BB, ensuring uniform concentration and volume across all samples. Mix briefly by vortexing and centrifuge with pulse spin. Then, heat the sample at 95°C for 2 minutes, followed by immediately cooling on ice.

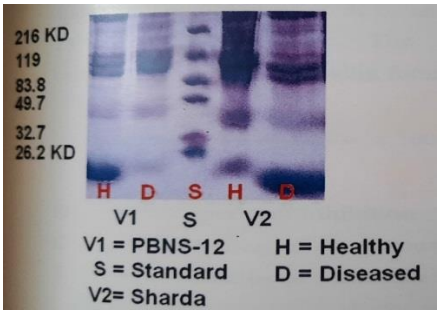
Gel Electrophoresis: Gel loading and running

The upper chamber of electrophoresis apparatus is filled with 1x Tris-glycine gel buffer.
Using a 10 µL pipette all sample is loaded into well carefully.10 µL Benchmark Pre stained Ladder (Invitrogen 10748-010) is loaded into in one lane. The lower chamber with 1x Tris-glycine gel buffer. The gel is run at 200 V for 55 minutes.

Staining the Gel

The gel is fixed, stained and destained according to the manufacturer's instructions for Brilliant Blue G-colloidal concentrate (Sigma, B2025).
NOTE: Mini gels require about 50 mL solution to cover a square Petri dish (100mm x 100mm).

Fix the gel by submerging it in a solution containing 7% glacial acetic acid and 40% methanol (v/v) for 30 minutes with gentle agitation. Dispose of the used fixing solution by transferring it to the designated methanol waste container. Prepare staining suspension by combining 40 ml of Brillent blue G--colloidal concentrate, 10 ml. methanol, and then apply it to the gel. Allow ti to rock gently for 1-2 hours or overnight as desired. Discard the staining solution by pouring it into the methanol waste container. Treat the gel with Destain solution 1 (10% acetic acid in 25% (v/v) methanol) while shaking vigorously for 10-30 seconds. Dispose of the destain solution by pouring it into methanol waste container. Wash the gel with 25% methanol (Destain solution II). Soak the gel with 25% methanol (Destain solution II) to remove excess stain for maximum 24 hours. Pour the 25% methanol into designated methanol waste container. Any residual dye precipitate on the gel surface, delicately clean it using a lint-free lab wipe or cotton wool dampened with methanol. Hydrate the gel by adding MilliQ water to it. Continue with the analysis of the gel.



Result and Discussion:

Identification of the banding pattern of protein differed in band size and staining intensity. Total seed protein was separated by SDS-PAGE method. The molecular masses ranged from 26.2 KD to 216 KD. The significant difference was recorded in seed protein content in healthy and infected seeds of both varieties. In variety PBNS- 12 the significant difference in seed protein was occurred in region 26.2 KD to 32.7 KD and 119 KD to 216 KD where as in variety Sharda the significant difference was observed in region 119 KD to 216 KD. While rest of other regions show similar banding pattern in both the varieties of healthy and infected seeds (Plate 1).

Jirair crapetain and Gholamereza Zarel (2005) and recorded that the amount of fatty acid protein in wild species was higher than cultivated species. Variation in protein, oil content of three wild species of safflower was studied. Akbar et.al. (2012) used sodium Dodecyl sulfate polyacrylamide Gel electrophoresis (SDS-PAGE) technique to study genetic diversity of sesamum indicum for total seed protein. Zeda et.al. (2013) reported SDS-PAGE method to study protein based genetic diversity in *Brassica carinata*. Nisar et.al (2011) Zabta khan shinwari et.al (2014) reported the size of protein bands ranges from 5 to 232 KD A. Zilic et.al (2010) reported seed protein profiling pattern in sunflower protein bands. Khurshid & Rabanni (2012) employed SDS-PAGE for electrophoretic comparisons of seed protein profiles from seeds of different Brassica cultivars This technique separates the seed storage proteins of crop. Nisar et al.(2011).

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Conflicts of interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

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