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Quantitative and qualitative analysis of soluble seed protein in okra [*Abelmoschus esculentus* (L.) Moench]

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ABSTRACT

Okra, a warm-season vegetable crop, is a significant source of vitamins, proteins, and minerals. It occupies the fifth position in terms of area and production, with a significant share of okra seed in the domestic and international markets. Seed quality is assessed based on physiological and biochemical parameters, such as protein, carbohydrate, fat, and enzymes. The quantity of seed protein is a strong determinant of seed quality, as it acts as a storage reserve of nitrogen, carbon, and sulphur. Seed protein analysis during development and maturation reveals changes in protein content, composition, and quality. These proteins are crucial for seed viability, growth, and germination. In the present experiment, soluble protein in seeds showed an increasing trend, followed by rapid accumulation at 30 days after anthesis and 35 days after anthesis in pre and post-kharif season respectively. The maximum protein content is recorded at 40 days after anthesis for both growing seasons. Differentiation in banding patterns and developmental stages helps distinguish genotypes. The major difference in banding pattern between 14 kD-45 kD was observed, and developmental stages 25 days after anthesis and 30 days after anthesis were found suitable for genotype differentiation based on SDS-PAGE protein profiling.

Key words: Okra, protein profiling, SDS PAGE, gel electrophoresis, biochemical parameters.

INTRODUCTION

Okra [*Abelmoschus esculentus* (L.) Moench] is an important warm season vegetable crop belonging to the family *Malvaceae*. Its tender fruit used for vegetable purposes which is commonly known as ladies 'fingers. It is also an excellent source of vitamins A and B, protein and minerals. India is the largest producer of okra globally, with a contribution of more than 72 percent (6 million tonnes) from an area of 0.5 million hectares (NHB, 8). Okra has vast potential for earning foreign exchange as it has a significant share in fresh vegetable export (APEDA, 2). Due to this there was a significant share of okra seed in the domestic and international markets. Availability of quality seed in any crop is a major concern to reap the high production. Assessment of the quality of seed is done basically on physiological parameters. The seed achieve its potential level of vigour and viability gradually during three distinct phases of development viz., cell division, filling and maturation. Sahoo *et al.* (10) seeds of most crops attain high germination and vigour at a stage of maximum accumulation of dry mass indicating its physiological maturity. This is also manifested at the biochemical level such as the accumulation of seed biomolecules such as protein, carbohydrate, lipid etc. There were several studies that which revealed seed quality can also be assessed on biochemical parameters such as estimation of seed biomolecules (protein carbohydrate, fat and enzymes). The progression of seed filling is a highly complex and involves a number of enzymes especially for regulation of the storage components. Among the seed reserves, the quantity of seed protein is a strong determinant of seed quality. Several studies revealed that quantity of seed protein available in seed is highly correlated with-it germination and vigor. Its main function is to act as a storage reserve of nitrogen, carbon, and sulphur. These proteins are rapidly mobilized during seed germination and serve as the major source of reduced nitrogen for the growing seedlings. According to Balasubramanian and Sadasivam (3), the rate of protein accumulation was greatest between days 35-42 days after anthesis. Changes in the protein profile during seed development and maturation can be tracked across developmental stages through SDS-PAGE (Singh *et al.,* 11). SDS-polyacrylamide gel electrophoresis is a common method used to separate proteins based on their molecular weight. In the present experiment, soluble seed protein profiling was conducted using SDS-PAGE. The success of electrophoretic procedure depends on wide ranging polymorphism of seed protein that represent primary gene product. These proteins banding pattern is unique for particular genotype (Kamel *et al.,* 6). So,

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it can be also used for genotype identification in many crops through electrophoretic analysis. The crieteria for distinctness among genotypes were the presence or absence of a particular bands occurring at a position on the electrophoretic gel. SDS-PAGE was used by Hassan *et al.* (5) for establishing the genetic identity in *triticum* species. Similar results were found by Kamel *et al*. (6) in Cruciferae, and Kumar *et al.* (7) in *Brassica juncea*. Singh *et al.* (12) also observed the electrophoretic variation in seed protein in the region of molecular weight 22 -27 kD across different cotton lines, likely to understand genetic diversity and potential nutritional or industrial applications. With the development of large number of okra cultivars, its identification is essential to maintain genetic purity during seed multiplication and certification. Therefore, quantitative and qualitative estimation of seed proteins accumulated gradually during seed development and maturation is need to be studied to find its variable pattern among the genotypes and growing season.

MATERIALS AND METHODS

Seed collected for eight genotypes of okra from various sources were grown in Randomized Block Design (RBD) at C-Block Farm (BCKV), Kalyani (Nadia), West Bengal, during pre-kharif and post kharif-2021-22 & 2022-23. The recommended agronomical package and practices were adopted to maintain the crops during the growing period. The developing capsules of the tagged flower were harvested at specific stages of seed development viz.,10, 20, 25, 30, 35, and 40 days after anthesis (DAA) for quantitative and qualitative analysis of soluble protein in seed. The seed collected at each stage of seed development was used for quantitative estimation of protein through lowery method. The protein profiling was also performed for qualitative estimation using SDS-PAGE. For extraction of soluble protein, 01 gm seed sample were ground with 0.1M phosphate buffer (pH 7.0) in a pre-cooled mortar and pestle. The homogenate was centrifuged at 10000rpm at 4°C for 30 minutes (Lowery *et al.,* 10). Then supernatant was collected for estimation of protein. The same protein sample was further resolved on 12.0 percent SDS polyacrylamide gel (Table 1). After the gel electrophoresis process was completed, removed the gel and it was washed to remove excess of SDS and stained for overnight. The staining solution contained 0.1 percent Coomassie brilliant blueR-250 in methanol, acetic acid and double distilled water with a ratio of 40:10:50. The gel was destained by using methanol, acetic acid and double distilled water without dye. The methods described by Valizadeh (14) were followed for SDS-PAGE protein banding pattern analysis. A standard marker as a ladder named Hi Range 2 protein marker-99625 (14-220 kDa) supplied by Sisco Research Laboratoty Pvt. Ltd. BioLit® was used.

RESULTS AND DISCUSSION

Seed protein accumulation is a crucial process during seed development and maturation, where storage proteins are synthesized and deposited to provide essential nutrients for the developing embryo and subsequent germination. In the present experiment, mean value shows a significance difference in the soluble protein content (mg g^{-1} of fresh weight of seed) in seed at various stages of seed development. Maximum protein content was recorded at 40 days after anthesis during both pre kharif and post kharif. However, non-significant difference was observed at 35 and 40 days after anthesis during pre-kharif. The mean value of soluble protein content at 40 days after anthesis was 52.85 mg and 56.80 mg during pre-kharif and post kharif respectively (Fig. 2). Maximum value was estimated in Kashi Kranti (33.41 mg) and Kashi Pragati (27.38 mg) during pre-kharif and post kharif respectively. Pattern of the accumulation of protein showed that in the early stages of seed development, the protein content tends to be low. During the mid stages the seed filling phase, storage proteins start to accumulate in larger quantities, particularly. Deposition and aggregation

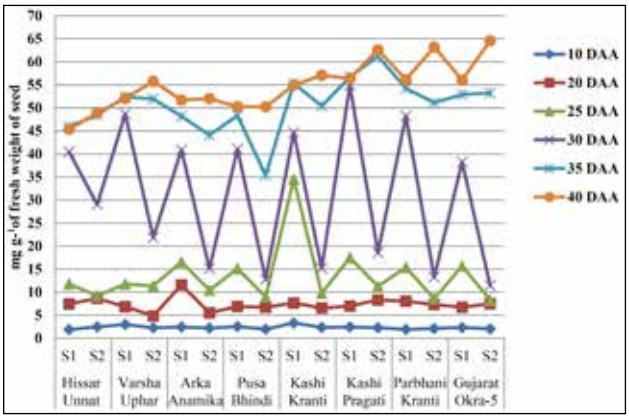
Table 1: Composition of resolving and stacking gel.

Resolving gel			Stacking gel		
S.N	Components	15.0ml	S.N	Components	5.0ml
1	H_2O	4.9ml	1	H_2O	3.4ml
2	30 % Acrylamide	6.0ml	2	30 % Acrylamide	0.8ml
3	1.5M Tris (pH 8.8)	3.8ml	3	0.5M Tris (pH 6.8)	0.63ml
4	10% SDS	0.150ml	4	10% SDS	0.05ml
5	10% APS	0.150ml	5	10% APS	0.05ml
6	TEMED	0.01ml	6	TEMED	0.01ml

of this protein supports the energy needs of the seed during germination as well as associated with the desiccation tolerance in seed (Vijay *et al.,* 15). Balasubramanian and Sadasivam (3) found that the rate of protein deposition was greatest between 35 days after anthesis to 42 days after anthesis in the okra cultivar Pusa Swami. Similar pattern of accumulation was also reported by Sridevi and Manonmani (13) in Prosso millet (*Panicum miliaceum* L). genotypic difference in protein content was also observed during both seasons. In an interaction effect, there was a significant demarcation in the development stages with the genotypes. Maximum soluble protein was estimated in Kashi Pragati (56.71 mg) at 35 days after anthesis during pre-kharif and found at par with the value at 40 days after anthesis of the same genotype. Similarly, the genotype Gujarat Okra-5 (64.58 mg) showed the highest soluble protein content at 40 days after anthesis in post kharif. The interaction of genotype and developmental stage showed non-significant interaction for most cases in between 35 days after anthesis and 40 days after anthesis. However, the maximum interaction values showed significant demarcation among them for the both seasons.

Qualitative analysis of soluble seed protein

The seed sample collected for quantitative analysis of soluble protein in seed at different stages of its development was further evaluated qualitatively through SDS PAGE (Table 2) as prescribed by Valizadeh (14) to assess the variation in the banding pattern of individual genotypes as well as a trend in change of the banding pattern with progress in seed development (Fig. 1). Number of protein bands was appeared and recognized with the advancement of the seed developmental stage ranging from 14 kD to 166 kD. Variation in a number of bands among



	Pre Kharif (S_1)			Post Kharif (S_2)		
	G	D	G × D	G	D	G × D
SEm(±)	0.07	0.06	0.18	0.06	0.05	0.15
CD(P=0.05)	0.20	0.18	0.50	0.17	0.14	0.41

G-Genotype; D-Stage of Development; S-Season

Fig. 1. Influence of season on genotype for soluble protein in seed during its development

Table 2: SDS PAGE banding pattern of soluble seed protein of okra during pre-*kharif*.

Band Size (kD)	Stage of Development					
	10 DAA	20 DAA	25 DAA	30 DAA	35 DAA	40 DAA
220	-	-	-			
116	-	-	-	G_1, G_2, G_3, G_4, G_5, G_6, G_7, G_8	G_1, G_2, G_3, G_4, G_5, G_6, G_7, G_8	G_1, G_2, G_3, G_4, G_5, G_6, G_7, G_8
95	-	-	-	-	-	-
66	-	G_5	G_1, G_2, G_3, G_4, G_5, G_6, G_7, G_8	G_1, G_2, G_3, G_4, G_5, G_6, G_7, G_8	G_1, G_2, G_3, G_4, G_5, G_6, G_7, G_8	G_1, G_2, G_3, G_4, G_5, G_6, G_7, G_8
45	-	G_5		G_5, G_6, G_7	G_5, G_6, G_7	G_5, G_6, G_7
35	-	G_2	G_1, G_2, G_4, G_5, G_6, G_7, G_8	G_1, G_2,G_4, G_5, G_6, G_7, G_8	G_1, G_2,G_4, G_5, G_6, G_7, G_8	G_1, G_2, G_3, G_4, G_5, G_6, G_7, G_8
25	-	G_2,G_5	G_2, G_4, G_5, G_6, G_7	G_2, G_4, G_5, G_6, G_7	G_2, G_4, G_5, G_6, G_7	G_1, G_2, G_3, G_4, G_5, G_6, G_7, G_8
22	-	G_5	G_1, G_2, G_3, G_4, G_5, G_6, G_7, G_8	G_1, G_2, G_3, G_4, G_5, G_6, G_7, G_8	G_1, G_2, G_3, G_4, G_5, G_6, G_7, G_8	G_1, G_2, G_3, G_4, G_5, G_6, G_7, G_8
20	-	G_5	G_1, G_2, G_3, G_4, G_5, G_6, G_7, G_8	G_1, G_2, G_3, G_4, G_5, G_6, G_7, G_8	G_1, G_2, G_3, G_4, G_5, G_6, G_7, G_8	G_1, G_2, G_3, G_4, G_5, G_6, G_7, G_8
14	-	-	G_1, G_5	G_1, G_5,G_6	G_1, G_5,G_6	G_1, G_4, G_5, G_6, G_7

G_1- Hissar Unnat G_2- Varsha Uphar; G_3-Arka Anamika; G_4-Pusa Bhindi-5; G_5-Kashi Kranti; G_6-Kashi Pragati; G_7-Parbhani Kranti; G_8-Gujarat Okra-5; DAA: Days after Anthesis

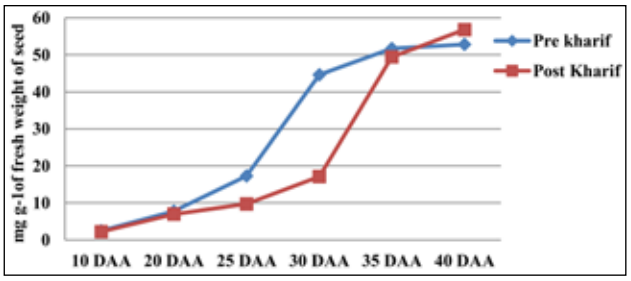


Fig. 2. Accumulation pattern of soluble protein in seed during pre-kharif and post kharif.

genotypes at specific was evident through the gel picture (Fig. 3 and 4). The protein profile at 10 DAA did not show any specific band for none of the genotypes. The presence of bands was recorded at 20 DAA for two genotypes only. i.e. Hisar Unnat (25 kD and 35 kD) and Kashi Kranti (66 kD, 45 kD, 25 kD, 22 kD and 20 kD). The presence of a specific additional band was recorded for all genotypes at 25 DAA. Three protein profiles considering 66 kD, 22 kD and 20 kD were present in all genotypes. Newly appeared profile of 45 kD was reported in Kashi Pragati and Parbhani Kranti which was absent in the previous developmental stage. Band size of 35

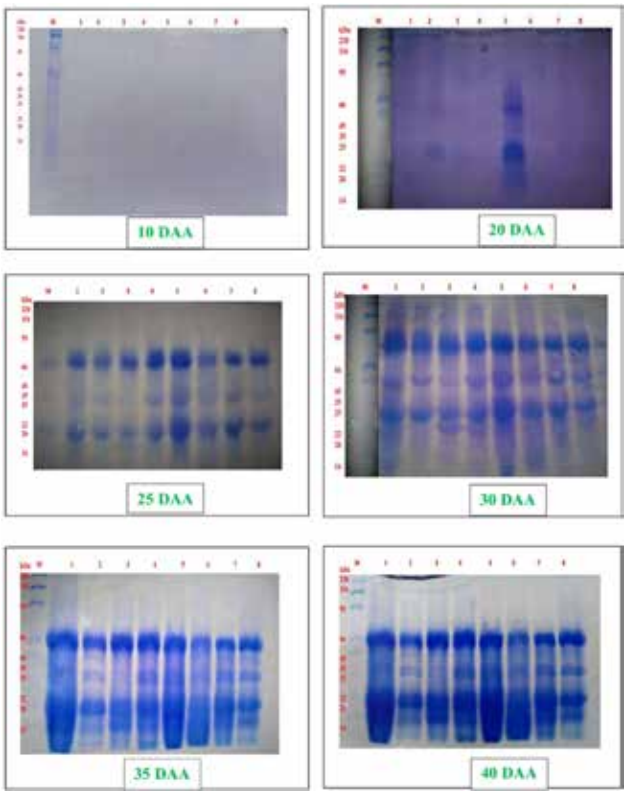


Fig. 3. Protein profiling during pre kharif at seed developmental stage.

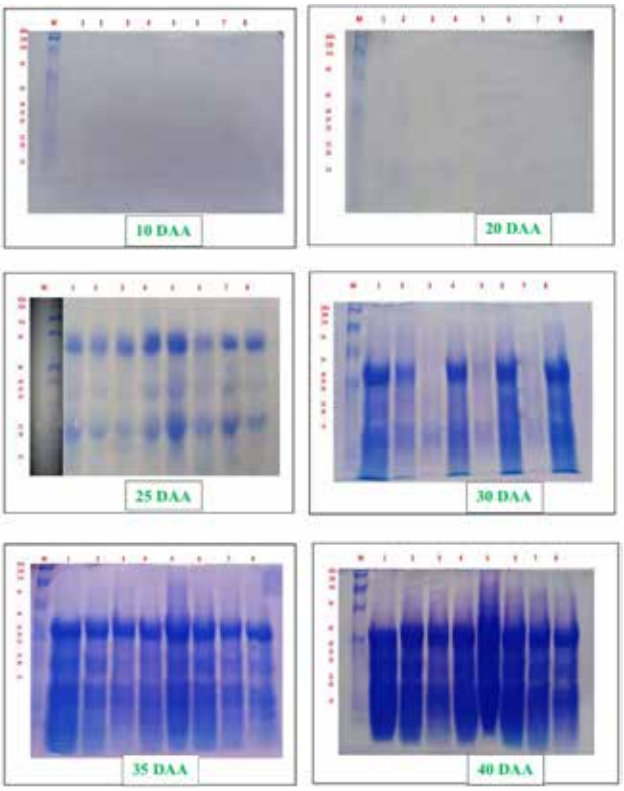


Fig. 4. Protein profiling during post kharif at seed developmental stage.

kD appeared in all genotypes except Arka Anamika. Similarly, new band of size 25 kD was reported in all genotype except Varsha Uphar and Arka Anamika. At seed development, the protein band of 14 kD was scored in genotypes, Varsha Uphar and Kashi Kranti. At next stage i.e. 30 days after anthesis, additional band size of 166 kD were appeared in all genotypes. The polypeptide band size of 45 kD, 35 kD, 25 kD, 22 kD and 20 kD were represented in all genotypes similar to earlier observation of 25 days after anthesis. The polypeptide band of 14 kD, it was additionally appeared in Kashi Pragati. The protein banding pattern at 35 days after anthesis was found very similar to reported in 30 days after anthesis. Additional polypeptide band of size 14 kD appeared in genotype Pusa Bhindi-5 and Parbhani Kranti at 40 days after anthesis. But the other banding pattern was the same as reported in 35 days after anthesis stage of development.

Similarly, method was also adopted for qualitative analysis of soluble seed protein during post kharif (Fig. 2). It was found that none of the genotype having specific band during seed development at 10 days after anthesis and 20 days after anthesis. Banding pattern was observed at 25 days after anthesis and onward stages of development. Polypeptide

band of size 66 kD, 45 kD, 35 kD, 22 kD and 20 kD were resolved in all the genotypes. Other than Varsha Uphar, Arka Anamika and Gujarat Okra-5, all genotypes were scored for band size 25 kD. In progression of seed development, a few additional bands were reported to get resolved. Profiling of soluble seed protein at 30 days after anthesis showed the appearance of new band like 116 kD and 14 kD. The 116 kD band was scored for all genotypes except Varsha Uphar and Parbhani Kranti, whereas 14 kD band was observed for all genotypes except Hisar Unnat, Arka Anamika and Parbhani Kranti.

At the growth stage of 35 days after anthesis, an additional band of 116 kD in Parbhani Kranti was reported whereas this band size was not appeared in others. Similarly, the band size of 35 kD did not appear in Arka Anamika, similar to previous developmental stages. In the banding pattern at 35 days after anthesis, all the genotypes were scored particularly positive for a band size of 14 kD. Further, soluble seed protein banding pattern at 40 days after anthesis, all genotypes scored positive for band size 116 kD, 66 kD, 45 kD, 35 kD, 25 kD, 22 kD, 20 kD and 14 kD, but no indication was found for 95 kD and 220 kD. Evidence from the present experiment showed that the selected genotypes were tested in twice on the same land under different environmental conditions i.e. during pre-kharif and post kharif. The seeds were collected for protein profiling at different stages of seed development. It was observed that slight variations in a banding pattern against eight marker bands i.e. 116 kD, 66 kD, 45 kD, 35 kD, 25 kD, 22 kD, 20 kD and 14 kD, among genotypes and also at their development stages. On comparing the protein profiling of the genotypes, variation in the banding pattern was observed at respective stage of seed development during pre-kharif and post kharif. None of the bands was scored in any genotypes at 10 days after anthesis during the growing seasons. Similarly, no band was present except in the case of Kashi Kranti during pre-kharif at 20 days after anthesis. This showed the presence of a significant level of soluble protein in seed at this stage during pre-kharif only whereas it was delayed during post kharif. Difference in band of 45 kD and 35 kD during pre-kharif and post kharif was recorded at 25 days after anthesis but all other band appeared were similar in respective genotype during both seasons. During pre-kharif 45 kD polypeptide band was scored for all genotypes at 25 days after anthesis where as it was recorded only in Kashi Kranti, Kashi Pragati and Parbhani Kranti. Here also delay in gel expression of protein bands observed during post kharif. In contrast to this, a band size of 35kD was present in all genotypes at this stage of development except in the case of Arka Anamika during pre-kharif. Thus, banding pattern of the different okra genotypes clearly revealed the appearance of certain bands is specific to the genotype and the stages of seed development and these bands may be polymorphic in nature. Further, on the basis of the presence and absence of a specific band we can also examined the okra genotype for its identification purpose. The polymorphic bands are also very useful due to their appearance in some genotypes and contrarily absent in others. These bands can be used for the identification of various genotypes (Abdou, 1). Similar study was also conducted by Valizadeh (14) reported that seed protein is highly polymorphic and environmental influence on their electrophoretic pattern is limited. Similarly, Gustine *et al*. (4) found that seed protein markers are also used extensively in the characterization of varieties. The present investigation revealed variation in genotypes based on soluble protein profiles of seed (Table 3). The genetic affinities within cultivars of the same species generally corroborated the morphological analysis. Similar to our finding, the result of differentiation of yellow sarson and brown seeded types of *Brassica* clearly separated the yellow seeded and brown seeded varieties by SDS PAGE (Sadia, 9). However, we can conclude that, SDS-PAGE can reveal the differences among soluble proteins in okra genotypes (Fig. 5).

The study showed that soluble seed protein increased steadily during development, with rapid accumulation beginning around 30–35 days after anthesis and reaching a maximum at 40 days in both seasons. SDS-PAGE revealed clear variation in banding patterns among genotypes, stages, and seasons, particularly between 14–45 kD, with 25 and 30 days after anthesis emerging as the most informative stages for genotype differentiation. Overall, combined quantitative and qualitative protein analyses provided valuable insights

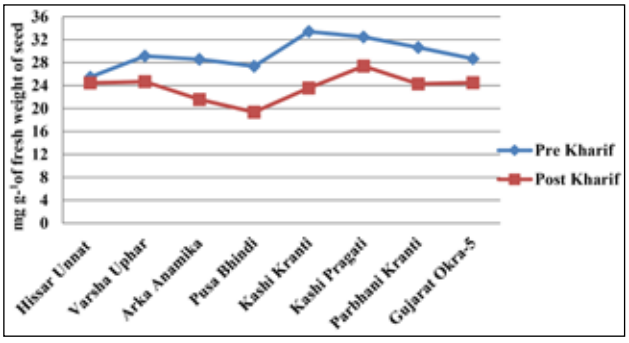


Fig. 5. Seasonal difference in average soluble protein in seed among genotypes.

Table 3: SDS PAGE banding pattern of soluble seed protein of okra during post kharif.

Band Size (kD)	Stage of Development					
	10 DAA	20 DAA	25 DAA	30 DAA	35 DAA	40 DAA
220	-	-	-			
116	-	-	-	G_2, G_3, G_4, G_5, G_6, G_8	G_2, G_3, G_4, G_5, G_6, G_7, G_8	G_1, G_2, G_3, G_4, G_5, G_6, G_7, G_8
95	-	-	-	-		
66	-	-	G_1, G_2, G_3, G_4, G_5, G_6, G_7, G_8	G_1, G_2, G_3, G_4, G_5, G_6, G_7, G_8	G_1, G_2, G_3, G_4, G_5, G_6, G_7, G_8	G_1, G_2, G_3, G_4, G_5, G_6, G_7, G_8
45	-	-	G_1, G_2, G_3, G_4, G_5, G_6, G_7, G_8	G_1, G_2, G_4, G_5, G_6, G_8	G_1, G_2, G_3, G_4, G_5, G_6, G_7, G_8	G_1, G_2, G_3, G_4, G_5, G_6, G_7, G_8
35	-	-	G_1, G_2, G_3, G_4, G_5, G_6, G_7, G_8	G_1, G_2, G_4, G_5, G_6, G_7, G_8	G_1, G_2, G_4, G_5, G_6, G_7, G_8	G_1, G_2, G_3, G_4, G_5, G_6, G_7, G_8
25	-	-	G_2, G_4, G_5, G_6, G_7	G_1, G_2, G_4, G_6, G_8	G_1, G_2, G_3, G_4, G_5, G_6, G_7, G_8	G_1, G_2, G_3, G_4, G_5, G_6, G_7, G_8
22	-	-	G_1, G_2, G_3, G_4, G_5, G_6, G_7, G_8	G_1, G_2, G_3, G_4, G_5, G_6, G_7, G_8	G_1, G_2, G_3, G_4, G_5, G_6, G_7, G_8	G_1, G_2, G_3, G_4, G_5, G_6, G_7, G_8
20	-	-	G_1, G_2, G_3, G_4, G_5, G_6, G_7, G_8	G_1, G_2, G_3, G_4, G_5, G_6, G_7, G_8	G_1, G_2, G_3, G_4, G_5, G_6, G_7, G_8	G_1, G_2, G_3, G_4, G_5, G_6, G_7, G_8
14	-	-	-	G_1, G_4, G_5, G_6, G_8	G_1, G_2, G_3, G_4, G_5, G_6, G_7, G_8	G_1, G_2, G_3, G_4, G_5, G_6, G_7, G_8

G_1- Hissar Unnat G_2- Varsha Uphar; G_3-Arka Anamika; G_4-Pusa Bhindi-5; G_5-Kashi Kranti; G_6-Kashi Pragati; G_7-Parbhani Kranti; G_8-Gujarat Okra-5; DAA: Days after Anthesis

into protein accumulation patterns during seed development, highlighting the influence of genetic and environmental factors on seed quality, viability, and germination.

AUTHORS' CONTRIBUTION

Formulation of research (MK); Development of the experimental design (MK & PC); Provision of experimental materials (MK & PC); Conducting of field/lab experiments and data collection (MK) Analysis and interpretation of data (MK & PC); Drafting of the manuscript (MK & PC)

DECLARATION

The authors declare that there is no conflict of interest

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