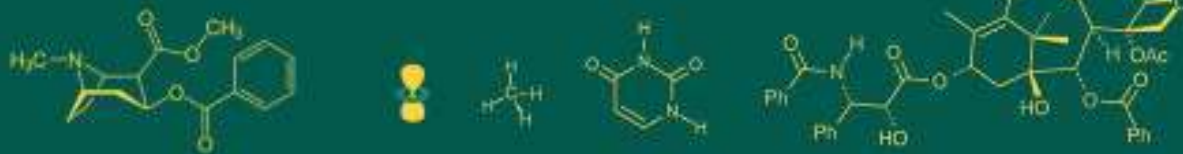


International Journal of Advanced Biochemistry Research



ISSN Print: 2617-4693
 ISSN Online: 2617-4707
 IJABR 2024; 8(10): 989-1004
www.biochemjournal.com
 Received: 03-09-2024
 Accepted: 02-10-2024

Pandit VM
 PG Student, Vilasrao
 Deshmukh College of
 Agricultural Biotechnology
 Latur, Maharashtra, India

Dhawale RN
 Research Guide, Vilasrao
 Deshmukh College of
 Agricultural Biotechnology
 Latur, Maharashtra, India

Bharose AA
 Associate Professor, Vilasrao
 Deshmukh College of
 Agricultural Biotechnology
 Latur, Maharashtra, India

Dudhare MS
 Associate Professor, Vilasrao
 Deshmukh College of
 Agricultural Biotechnology
 Latur, Maharashtra, India

Corresponding Author:
Pandit VM
 PG Student, Vilasrao
 Deshmukh College of
 Agricultural Biotechnology
 Latur, Maharashtra, India

Physiological and molecular characterization of ASR gene of pigeon pea (*Cajanus cajan* (L.) Millsp.) Genotypes under drought stress condition

Pandit VM, Dhawale RN, Bharose AA and Dudhare MS

DOI: <https://doi.org/10.33545/26174693.2024.v8.i10m.2660>

Abstract

The present study envisaged the physiological and molecular characterization of ASR gene of pigeon pea genotypes under drought stress condition. All the 12 pigeon pea genotypes were subjected to physiological characterization under drought stress condition for identification of ASR gene in the field in three replications under control, 06 days, and 12 days conditions during summer 2023-24. The data from summer 2023-24 was subjected to statistical analysis. The Analysis of variance (ANOVA) for drought stress tolerance or resistance index was performed for all 12 genotypes using RBD analysis software. ANOVA showed non-significant genotypic differences for drought stress tolerance or resistance index and also between replication there was no significant difference for all 12 pigeon pea genotypes shown in the result of the RBD analysis. There was an unknowingly less is more in all the parameters measured from day 0–12 of drought stress in all the genotypes with control. Control plants maintained a higher protein content (leaf) (A), Fresh shoot weight (B), Fresh weight (C), Turgid weight (D), Dry weight (E), and Shoot dry matter content (F) in comparison to drought stressed plants. In the current study, the analysis of physiological responses demonstrated that ASR gene expression in drought stress and in control condition neither improve nor reduced in water stress, which is helped to maintain studied physiological characters under water stress. A perusal of dendrogram indicated that an elucidian distance of 0.16 indicating that pigeon pea genotypes did not show much variation at DNA level and out of total 1775 amplicons polymorphism detected only 25.77% polymorphism with 9.61 average numbers of polymorphic bands. The designed primer(s) are perfect but not binding with the target and giving single sharp band on a DNA electrophoresis gel. It can be concluded that the parameters used for primer designing by Primer3 play a critical role in the experimental results and parameters defined in the present study resulting in a negative amplification during PCR of all 12 genotypes. Therefore, it was concluded that the 12 pigeon pea genotypes collected from ARS, Badnapur don't show the amplification of ASR gene. According to the comparative analysis results of all genotypes belongs to drought susceptible.

Keywords: *Cajanus cajan*, drought, physiological, ASR, RAPD, Badnapur

Introduction

[*Cajanus cajan* (L.) Millsp.] Pigeon pea (2n = 22) is a significant pulse crop that plays a significant role in Indian agriculture (Odeny 2007, Choudhary, *et al.*, 2013) [18, 4]. Pigeon pea seeds are consumed as a dry pulse or green vegetable and are a good source of ascorbic acid, protein, vitamin B, and carotene. Although pigeon peas are a self-pollinating crop, bee pollination raises productivity (Abrol and Shankar, 2015) [1]. The annual crop species known as pigeon pea is typically cultivated on marginal terrain with little to no inputs. There are five major maturity stages for pigeon pea: extremely early (95 days), extra-early (91–120 days), early (121–150 days), medium (161–200 days), and late (>250 days) (Sinha P. *et al.*, 2020) [25]. Rainfed pigeon peas are cultivated during the Kharif season (Basu T. K. and Bandyopadhyay S. R. 2009) [3]. The pigeon pea, a major grain legume crop in the tropics and subtropics, is a perennial member of the Fabaceae family. Pigeon pea is only ranked sixth in terms of area and production among grain legumes, but it is utilized in a wider variety of ways than other legumes. A resilient, adaptable, and drought-tolerant crop is pigeon pea (Qiao G. *et al.*, 2011) [20]. However, the pigeon peas' life and yield are seriously threatened by the environment they are in, which includes high temperatures, salt, a lack of water, and abrupt cold.

Therefore, it is increasingly crucial to research how the pigeon pea gene functions under stress (Zhihua Song, *et al.*, 2020) [26]. Many biotic and abiotic stressors, including infections, can impact crop plants. Abiotic stresses include salt, drought, extremes in the environment, and toxic substances. Crop production as well as quality have decreased substantially, as a result of these pressures that are greater. As a result, agricultural crops have to get improved to survive stressors both biotic and abiotic in nature (Kopecka, R. *et al.*, 2023) [14].

Many biotic and abiotic stressors, including infections, can impact crop plants. Abiotic stresses include salt, drought, extremes in the environment, and toxic substances. Crop production as well as quality have decreased substantially, as a result of these pressures that are greater. As a result, agricultural crops have to get improved to survive stressors both biotic and abiotic in nature (Kopecka, R. *et al.*, 2023) [14].

Throughout the plant life cycle, abscisic acid (ABA) is crucial for controlling a number of aspects of growth and development. During vegetative growth, ABA is especially important for enabling plants to detect and react to a variety of unfavourable environmental stresses, such as drought, salt, and cold. (Leung and Giraudat, 1998; Finkelstein, *et al.*, 2002) [15, 8]. The great hydrophilicity of the ASR proteins makes them tiny and heat-stable proteins (Silhavy, *et al.*, 1995; Frankel, *et al.*, 2006) [24, 9]. According to reports, ASRs belong to the well-recognized class of hydrophilins, which also includes the late embryogenesis abundant (LEA) proteins that are particular to seeds. (Dure, *et al.*, 1989) [6]. It was found that the first ASR protein was a tomato protein produced by ABA and water stress. (Iusem, *et al.*, 1993) [12]. Understanding the molecular processes by which plants respond to such environmental challenges has been the subject of much research. Significant alterations in the expression patterns of many gene classes are triggered by high salinity and water deficiency, with the majority of these genes being linked to ABA signalling pathways (Rabbani *et al.*, 2003; Shinozaki and Yamaguchi Shinozaki, 2007) [21, 23]. Land immigration by species throughout the Paleozoic period has caused these plants to adopt many modification techniques to withstand desiccation (Edwards and Selden, 1992) [7]. The ASR family's diversity study has demonstrated how crop plants have undergone adaptive selection to help them adapt to their surroundings. The transcription factors belonging to the Asrgene family-abscisic acid, stress, and ripening-induced stress-are peculiar to plants and are controlled by stress in the ABA-dependent pathway. Their interaction with ABRE elements provides additional evidence of their significance. According to reports, the ASR gene family plays a major role in how plants react to various developmental and environmental stressors that require ABA signaling, including

environmental abiotic stresses. (Mathlouthi, N. *et al.*, 2022) [17]. Studies on subcellular fractionation also demonstrated that the cytoplasm and nucleus contain ASR protein. Strong selection pressures and reduced gene diversity were seen in the accessions tested regarding the possible function of the ASR1 gene in drought tolerance in common beans. (Sachdeva, S. *et al.*, 2020) [22]. The conserved ABA/WDS domain (PF02469) is a defining characteristic of a small gene family that includes the abscisic acid-, stress-, and ripening-induced (ASR) genes (Hamdi, K. *et al.*, 2020) [10]. ASR genes are noticeably lacking in the model plant *Arabidopsis*, despite the fact that this gene family has been found in a number of plant species, including rice, tomato, and maize. At the N and C termini of each ASR protein, respectively, is a zinc-binding domain and a potential nuclear targeting signal (Jin, S. Y. *et al.*, 2021) [13]. ASR proteins have been linked to the response to several abiotic stressors, including osmotic stress, salt stress, and water deficiency, on multiple occasions. Numerous plants, including rice, maize, tomatoes, potatoes, maize, or rice. A zinc-dependent DNA-binding domain is also present in certain ASR proteins, and it has been shown that these proteins can bind DNA both in a homodimeric and monomeric form (Isabelle M. Henry *et al.*, 2011) [11].

Materials and Methods

The present experiment was carried out at Department of Plant Biotechnology, Vilasrao Deshmukh College of Agricultural Biotechnology, Latur. A constituent college of Vasantrao Naik Marathwada Krishi Vidyapeeth, Parbhani during the year 2022-23. Selected twelve pigeon pea (*Cajanus cajan* (L.) Millsp.) genotypes was grown in pots with drought stress treatment. All normal packages of practices were applied to raise a crop for good sampling. Pot experiment were conducted at Vilasrao Deshmukh College of Agricultural Biotechnology, Latur, Maharashtra during the summer season (March - April) in the 2024 to study responses under well-watered, i.e., irrigated and drought stress conditions. The experiment was conducted in a pot size of 1m² with 3 rows in a randomized block design (RBD) with 3 replications (fig 1). Fertilizer was applied for the crop. The irrigated experiment served as a control where need irrigation was given and drought stress experiment where no water was applied. Drought stress is imposed on 12 days old seedlings by withholding water for 6 days, 12 days respectively.

Physiological data analysis

The analysis of variance (ANOVA) for physiological was carried out using RBD analysis. PDI from two replications was subjected to ANOVA. Means followed by different letters within a column are significantly different from each other according to Tukey's Studentized Range (HSD) test.

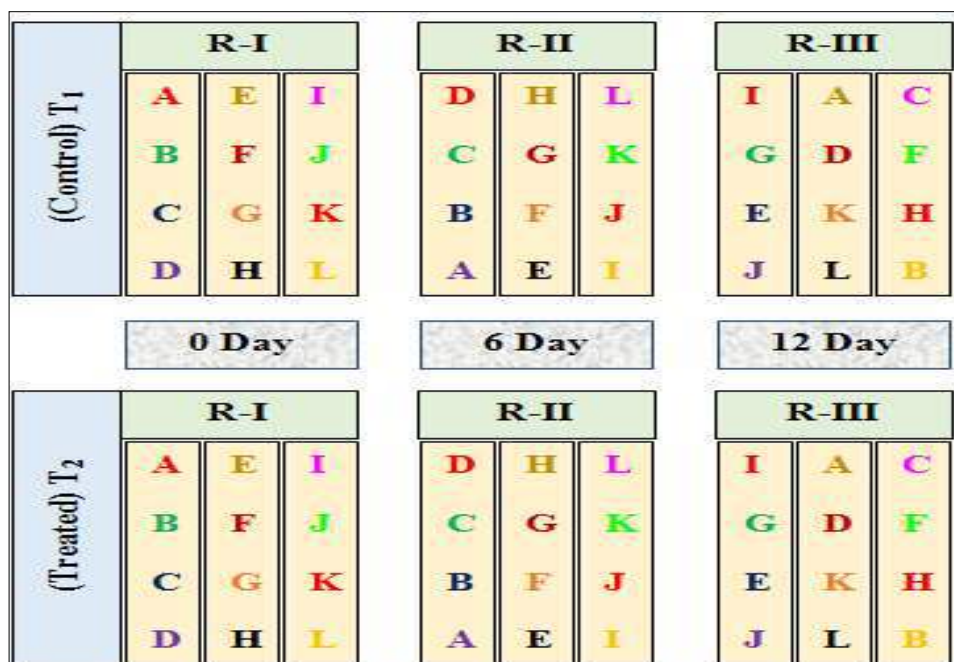


Fig 1: The experiment was conducted in a randomized block design (RBD) with 3 replications



Fig 2: Drought imposed in pot experiment of pigeon pea genotypes in RBD

Relative water content

1. Relative water content (RWC) was measured on the third and fourth fully expanded leaves from the top of the main stem from four plants in each subplot between 10.00 a.m. and 12.00 p.m. on sunny days.
2. Two leaves per plant were cut into small pieces with a disc borer of 2.0 cm². The leaf samples were put into zipped polyethylene bags and placed immediately in a cooler box to prevent moisture loss.
3. Leaf fresh weight (FW) was recorded by weight as soon as possible.
4. Turgid leaf weight (TW) was recorded after immersion into distilled water under controlled room temperature at 25 °C under dim light for 8 h, and then, dry weight was recorded after oven drying at 80 °C for at least 72 h or until the weight was constant.

RWC was calculated based on a formula (Aunchana Chaimala *et al.*, 2020) ^[2]

$$RWC = \frac{(FW - DW)}{(TW - DW)} \times 100$$

Protein content

The Protein content from the pigeon pea fresh leaves using Lowry method developed and standardized by Lowery *et al.*, (1951). For estimating protein content (leaf) at different time points, crushing of leaves was done in 50mM phosphate buffer with pH 7.8. The protein content was estimated by colorimetric method. Absorbance of the samples was recorded on spectrophotometer at 600 nm. The Bovine Serum Albumin was used as a standard. The protein content was expressed in µg ml⁻¹.

1. Weight 1 gram of leaf sample and add 1 ml of phosphate buffer in cold mortar and pestle.
2. Grind the leaf sample in phosphate buffer and transfer homogenized mixture in 1ml of centrifuge tube.
3. Incubate the homogenized sample for 2 hrs. and then centrifuge at 12000 rpm.
4. After centrifugation 2 layers are form that was supernatant and precipitation. Then transfer supernatant into separate tube.
5. 0.2, 0.4, 0.6, 0.8, 1ml of BSA working standard in 5 test tubes and make up to 1ml using distilled water.
6. The test tube with 1 ml distilled water serve as blank.
7. Add 4.5 ml of reagent I and incubate for 10 minutes.
8. After incubation add 0.5 ml of reagent II and incubate for 30 minutes
9. Measure the absorbance at 600 nm.

Shoot dry matter content

The Shoot dry matter contents the pigeon pea fresh leaves using a procedure standardized by Aunchana Chaimala *et al.*, (2020) ^[2].

1. The plants in the border rows and 2 ends of the middle row were discarded, and 35 plants in an area of 5.25 m² of each subplot were cut at ground level and separated into leaves, stems, and tubers.
2. The samples were used to record the number of tubers per plant and fresh weights of all plant parts. Dry weights were recorded after those samples were oven-dried at 70 °C for 72 h or until weights were constant.

- Total biomass was calculated by the sum of stem, leaf, and tuber dry weight and shoot dry weight was computed by the sum of stem and leaf dry weight.

Weight of fresh shoot

As light and air temperature changes, shoot fresh weight is evaluated. (Masayuki ODA and Kenkoh Tsuji 1992) [16].

- Harvest the plant:** Remove the plant from the soil and wash off any loose soil.
- Remove excess water:** Gently blot the plant with a soft paper towel to remove any surface moisture.
- Weigh immediately:** Weigh the plant as soon as possible to avoid drying out and getting inaccurate data.

Isolation of plant genomic DNA: The genomic DNA extracted from the pigeon pea fresh leaves using a procedure standardized by Doyle J. (1991) [5]. Use this procedure with minor modification for DNA isolation.

Amplification of DNA gene by PCR: For DNA amplification, the genomic DNA, primers, 10X PCR buffer, dNTPs, and *Taq DNA polymerase* were all optimized. A master mixture consisting of 20.9 μ l of sterile H₂O, 2 μ l of

10 X PCR buffer (1.5 mM MgCl₂), 0.4 μ l dNTPs, 0.2 μ l of each primer (F and R), and 0.3 μ l of *Taq polymerase* was created for the 12 pigeon pea species reactions against a single primer. In a PCR tube, 1.0 μ l of diluted DNA from each pigeon pea was combined with 24.0 μ l of master mixture for a single sample reaction. In a thermal cycler, the DNA amplification process was carried out.

Results and discussion: All the 12 pigeon pea genotypes were subjected to physiological characterization under drought stress condition for identification of ASR gene in the field in two replications during summer 2023-24. There was no previous study of all 12 pigeon pea genotypes towards drought stress tolerance or resistance and it was studied to be non-significant during summer 2023-24. The data from summer 2023-24 was subjected to statistical analysis. The Analysis of variance (ANOVA) for drought stress tolerance or resistance index was performed for all 12 genotypes using RBD analysis software.

Pigeon pea genotypes under control and drought stress conditions Table 1 and Table 2.

Table 1: ANOVA for all the four physiological traits under study viz., protein content (leaf) (A), Fresh shoot weight (B), Fresh weight (C), Relative water content (Fresh weight, Turgid weight, Dry weight) AND(D), and Shoot dry matter content (F) in the twelve pigeon pea genotypes under drought stress conditions. Measurements were taken at 0 day (control conditions), 6th day after imposition of drought stress, 12th day after imposition of drought stress.

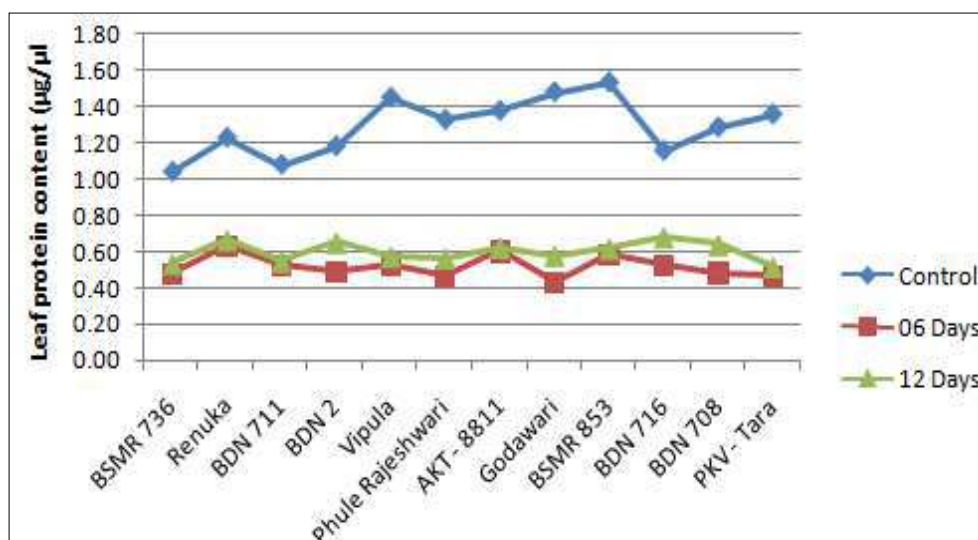
Source of Variation	df	Leaf protein content (μ g/ μ l)	Fresh shoot weight (gm)	Relative water content			Shoot dry matter content (Gm)	F crit
				Fresh weight (mg)	Turgid weight (mg)	Dry weight (mg)		
Treatment	2	6.551	1.357	4.635	61.043	0.806	2.331	3.082
Error	105	0.017	0.004	0.537	1.759	0.083	0.023	
F value		390.825	315.488	8.626	34.702	9.737	103.060	

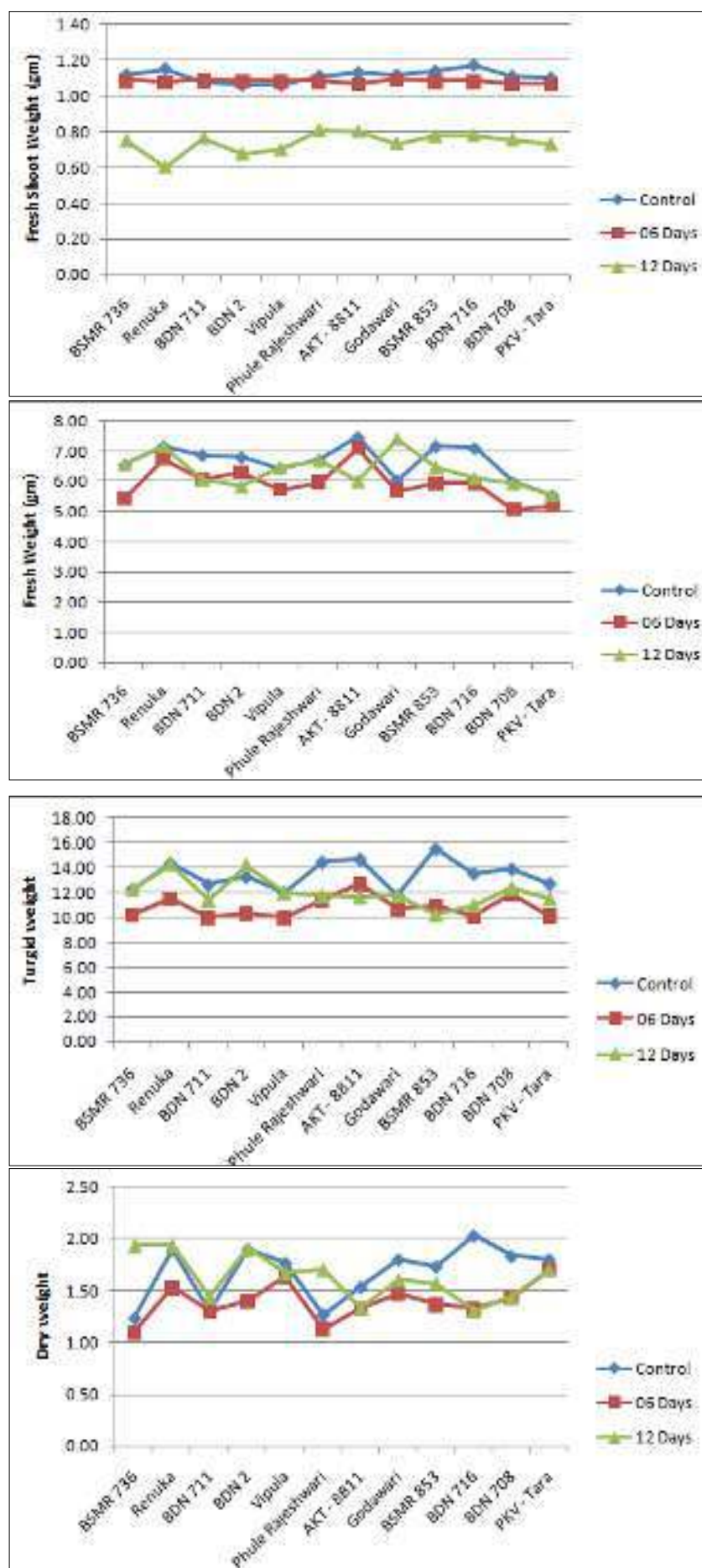
** Significance at $p \leq 0.05$

Table 2: ANOVA for all the four physiological traits under study viz., protein content (leaf) (A), Fresh shoot weight (B), Fresh weight (C), Turgid weight (D), Dry weight (E), and Shoot dry matter content (F) in the twelve pigeon pea genotypes under drought stress conditions. Measurements were taken at 0 day (control conditions), 6th day after imposition of drought stress, 12th day after imposition of control condition.

Source of Variation	df	Protein content (leaf)	Fresh shoot weight	Relative water content			Shoot dry matter content (gm)	F crit
				Fresh weight (mg)	Fresh weight (mg)	Fresh weight (mg)		
Treatment	2	0.076	0.008	0.990	4.279	0.224	0.060	2.015
Error	22	0.024	0.003	0.303	1.128	0.069	0.026	
F value		3.206	3.281	3.272	3.793	3.227	2.267	

** Significance at $p \leq 0.05$





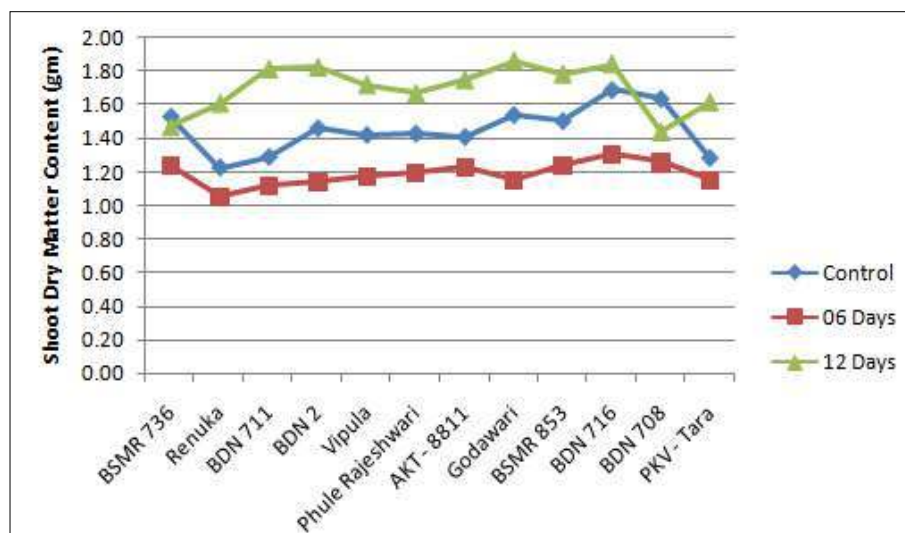


Fig 3: Pigeon pea genotypes

Relative Water Content (RWC)

The relative water content (RWC) of different crop varieties can be classified into high, medium, and low groups based on the values recorded under Control, 6 Days, and 12 Days conditions. This classification helps evaluate the crops' ability to retain water under stress conditions.

The high relative water content consistently demonstrates high relative water content in Godawari genotype, starting with the highest value at 56.84% under control, slightly dropping to 42.61% at 6 days, but recovering well to 50.86% by 12 days. This shows good water retention under stress. The BDN-2 starts with 55.26% in the control condition but show a noticeable decrease to 43.10% at 6 days and 40.89% by 12 days, although its initial value places it in the high category. The BDN-711 also has high water content, starting at 55.00% under control, declining to 46.12% at 6 days, but recovering to 49.49% by 12 days, reflecting good resilience. BDN-716 exhibits high RWC, the beginning at 52.46% in control, dropping to 44.06% at 6 days, and recovering to 49.48% by 12 days. The BSMR-853 starts at 51.73% in control, decreases significantly to 39.37% at 6 days, but recovers to 48.54% at 12 days, maintaining high overall water content. The medium relative water content observed in AKT-8811 starts with 50.73% under control, decreasing to 44.06% at 6 days, and then stabilizing at 45.01% by 12 days, placing it in the medium category. The Renuka exhibits medium RWC, beginning with 50.34% under control, dropping to 42.24% at 6 Days, but recovering somewhat to 47.32% by 12 days. The Vipula starts with 49.00% under control, decreases to 45.59% at 6 days, and maintains a medium level of 46.45% at 12 days. The Phule Rajeshwari starts at 46.93% in control, drops to 41.15% at 6 days, but recovers to 45.83% by 12 days, placing it in the medium range.

The low relative water content observed in BSMR-736 genotype and it starts with 47.61% under control, drops to 46.03% at 6 days, and continues to decrease to 44.84% by 12 days, making it one of the lower-performing crops in terms of water retention. The BDN-708 genotype shows low water content throughout, beginning at 46.71% under control, decreasing to a low 34.25% at 6 days, and recovering slightly to 41.03% by 12 days, reflecting poor performance. The PKV-Tara shows the lowest RWC, starting with 41.50% under control, dropping sharply to

34.24% at 6 days, and only recovering marginally to 39.11% by 12 days, indicating poor water retention.

Leaf Protein Content

The leaf protein content data can be categorized into high, medium, and low groups based on the values recorded under control, 6 days, and 12 days conditions. High leaf protein content BDN-708 consistently exhibits high protein content. Starting at 22.29% under control, it drops to 17.48% at 6 days but rebounds to a solid 18.63% at 12 days, indicating strong protein retention under stress. BDN-2 also shows a high initial content at 21.83% under control, although it decreases to 14.49% at 6 days, recovering somewhat to 16.65% by 12 Days. BDN-716 begins with a high protein content of 21.57% under control, but experiences a gradual reduction to 18.52% at 6 days and a more significant drop to 15.77% by 12 days. The genotype Godawari maintains high and stable protein content, starting at 19.48% under Control, remaining steady at 19.42% at 6 days, and even slightly increasing to 19.57% by 12 days. BDN-711 also shows high values, starting at 20.21% under control, decreasing to 17.52% at 6 days, but recovering to 19.54% at 12 days, displaying strong resilience. Medium leaf protein Content in PKV-Tara starts with a moderately high value of 20.36% under control, decreases slightly to 18.42% at 6 days, and ends with 17.51% by 12 days, indicating medium recovery. The Vipula begins with 20.53% under control, but experiences a notable decline to 15.53% at 6 days and only partially recovers to 16.57% by 12 days, placing it in the medium range. The Renuka starts at 19.12%, drops to 15.60% at 6 days, and recovers to 17.66% at 12 days, showing moderate recovery. The AKT-8811 starts with 19.38% under control, drops to 15.62% at 6 days, and recovers slightly to 17.62% at 12 days, demonstrating medium performance. The Phule Rajeshwari remains relatively stable throughout, starting at 17.73% under control, dropping slightly to 16.47% at 6 days, and recovering to 17.63% by 12 days, maintaining moderate levels. Low leaf protein content BSMR-853 starts strong at 20.37% under control, increases to 21.58% at 6 days, but shows a sharp decline to 15.61% by 12 days, placing it in the low category by the end. The BSMR-736 starts with 16.55% under control, decreases to 14.70% at 6 days, and shows almost no change, ending at 16.53% at 12 days. The

BDN-2, while initially high in the control condition, experiences a steep decline in leaf protein content, especially at 6 days (14.49%), making its recovery to 16.65% by 12 days relatively low in comparison to its starting point.

This categorization highlights how well the crops maintain or recover their protein content under stress conditions, with some showing significant declines and others exhibiting resilience. The high leaf protein content crops: BDN-708, BDN-2, BDN-716, Godawari, BDN-711. The medium leaf protein content varieties PKV-Tara, Vipula, Renuka, AKT-811, Phule Rajeshwari. The low leaf protein content varieties BSMR-853, BSMR-736, BDN-2 (Due to steep decline).



Fig 3: BSA standard for protein content

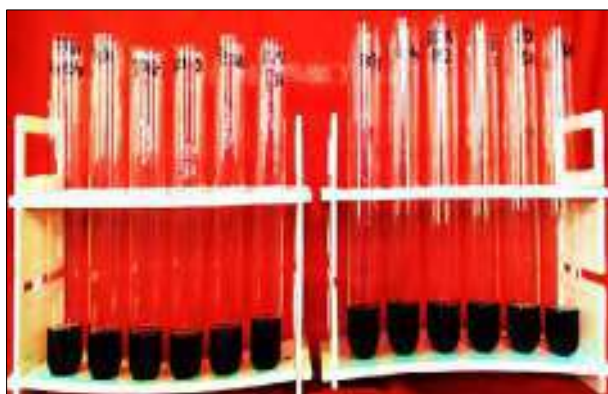


Fig 4: Genotypes sample of pigeon pea for protein content

Fresh Shoot Weight: The fresh shoot weight data for the crops can be categorized into high, medium, and low based on the weight values under control, 06 days, and 12 days conditions. Highest fresh shoot weight is observed BDN-716 consistently shows high values across all conditions, starting at 1.17 grams in control, decreasing to 0.89 grams at 06 days, and maintaining a relatively high value of 0.78 grams at 12 days. Phule Rajeshwari also falls into the high, with 1.11 grams under control, 0.85 grams at 06 Days, and 0.81 grams at 12 days, showing good resilience and minimal loss over time. BSMR 853 and Godawari show relatively high values in control (1.14 and 1.12 grams, respectively). The Godawari peaks at 0.90 grams at 06 days, although it falls to 0.74 grams by 12 days.

Among the all genotypes medium fresh shoot weight observed AKT - 8811 shows medium values across all conditions, was starting at 1.13 grams under control, dropping to 0.79 grams at 06 days, and slightly recovering to 0.80 grams at 12 days. BDN-2 and Vipula also fall into

the medium category, with similar trends: starting at around 1.06 grams in control, showing relatively stable weights at 06 days (0.83 grams for BDN 2 and 0.82 grams for Vipula), and ending at 0.68 grams and 0.70 grams, respectively, by 12 days. BSMR-736 starts at 1.12 grams under control, drops significantly to 0.67 grams at 06 days, and then recovers to 0.75 grams at 12 days, placing it in the medium and low fresh shoot weight seen in some genotypes Renuka exhibits low shoot weight values, starting at 1.15 grams under control, dropping to 0.78 grams at 06 days, and further decreasing to 0.60 grams at 12 days, showing poor recovery. BDN-711 shows similar low trends, with 1.08 grams in control, dropping to 0.67 grams at 06 days, and only slightly recovering to 0.77 grams by 12 days. BDN-708 and PKV-Tara fall in the low category as well, with 1.11 grams and 1.10 grams, respectively, in control. Both exhibit minimal change across the stress period, with BDN-708 remaining at 0.76 grams at both 06 and 12 days, and PKV-Tara stabilizing at 0.73 grams by 12 days.

This group highlights the variation in the varieties resilience and performance under stress, with some recovering well and others showing more significant declines. High fresh shoot weight varieties include BDN-716, Phule Rajeshwari, BSMR-853, and Godawari. Medium fresh shoot weight varieties include AKT-8811, BDN-2, Vipula, and BSMR-736. Low fresh shoot weight varieties include Renuka, BDN-711, BDN-708, and PKV-Tara (fig 4).

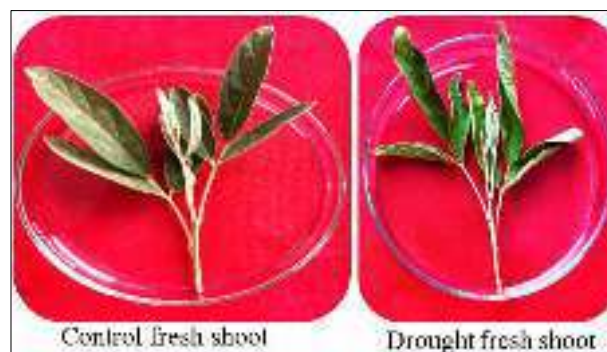


Fig 5: Fresh shoot weight of pigeon pea genotypes

Shoot Dry Matter Content

The shoot dry matter content for the crops can be classified into high, medium, and low categories based on their values under Control, 06 days, and 12 days conditions. Highest shoot dry matter content shows consistently high values in BDN-716, starting at 1.69 grams under control, slightly reducing to 1.31 grams at 06 days, and recovering to 1.84 grams at 12 days. It demonstrates resilience with a strong recovery. Godawari also falls in the high category, with 1.54 grams under control, dipping to 1.15 grams at 06 days, and recovering significantly to 1.86 grams at 12 days, indicating good performance. BDN-2 starts at 1.46 grams under control, decreases to 1.14 grams at 06 days, but shows a strong recovery to 1.82 grams at 12 days. BDN-711 exhibits a similar trend, beginning at 1.29 grams, slightly declining to 1.12 grams at 06 days, and then rebounding impressively to 1.81 grams by 12 days. Phule Rajeshwari starts at 1.63 grams in Control, decreases to 1.20 grams at 06 days, and recovers well to 1.77 grams at 12 days.

Among the some genotypes shows medium shoot dry matter content BSMR-736 begins with a shoot dry matter content of 1.53 grams under control, drops to 1.24 grams at 06

Days, and recovers to 1.47 grams at 12 Days, placing it in the medium range. BSMR-853 starts with 1.50 grams in control, dips to 1.24 grams at 06 days, and recovers moderately to 1.78 grams at 12 days, keeping it in the medium range. AKT - 8811 starts at 1.41 grams, dips to 1.23 grams at 06 days, and recovers to 1.75 grams by 12 days, showing moderate recovery. Vipula begins at 1.72 grams in control, drops significantly to 1.17 grams at 06 days, and only recovers partially to 1.42 grams by 12 days, indicating a medium recovery. BDN 708 shows a steady trend, starting at 1.64 grams in control, dipping slightly to 1.26 grams at 06 days, and recovering to 1.44 grams at 12 days. Remaining genotypes viz., Renuka shows the lowest Shoot Dry Matter Content exhibits low values, starting at 1.61 grams under control, dropping sharply to 1.06 grams at 06 days, and only slightly recovering to 1.22 grams at 12 days, indicating poor recovery. PKV-Tara falls in the low category, starting at 1.28 grams in control, dropping slightly to 1.16 grams at 06 days, and recovering moderately to 1.62 grams by 12 days, although the initial value was relatively low. This classification reflects the varieties resilience under stress conditions, with some showing strong recovery and

others experiencing significant reductions. High shoot dry matter content varieties BDN-716, Godawari, BDN-2, BDN-711, Phule Rajeshwari. Medium shoot dry matter content varieties like BSMR-736, BSMR-853, AKT-8811, Vipula, BDN-708. The low shoot dry matter content varieties are Renuka, and PKV-Tara.



Fig 5: Shoot dry matter content of pigeon pea genotypes

Table 3: Identification and grouping of pigeon pea genotypes based on protein content (leaf) ($\mu\text{g/ml}$), weight of fresh shoot (gm), relative water content (%) and shoot dry matter content (gm).

Genotypes	Protein content (leaf) ($\mu\text{g/ml}$)			Group	Weight of fresh shoot (gm)			Group
	Control	06 days	12 days		Control	06 Days	12 Days	
BSMR-736	16.55	14.70	16.53	Low	1.12	0.67	0.75	Medium
Renuka	19.12	15.60	17.66	Medium	1.15	0.78	0.60	Low
BDN-711	20.21	17.52	19.54	High	1.08	0.67	0.77	Low
BDN-2	21.83	14.49	16.65	High	1.06	0.83	0.68	Medium
Vipula	20.53	15.53	16.57	Medium	1.06	0.82	0.70	Medium
Phule Rajeshwari	17.73	16.47	17.63	Medium	1.11	0.85	0.81	High
AKT-8811	19.38	15.62	17.62	Medium	1.13	0.79	0.80	Medium
Godawari	19.48	19.42	19.57	High	1.12	0.90	0.74	High
BSMR-853	20.37	21.58	15.61	Low	1.14	0.82	0.78	High
BDN-716	21.57	18.52	15.77	High	1.17	0.89	0.78	High
BDN-708	22.29	17.48	18.63	High	1.11	0.76	0.76	Low
PKV-Tara	20.36	18.42	17.51	Medium	1.10	0.75	0.73	Low
Genotypes	Relative water content (%)			Group	Shoot dry matter content (gm)			Group
	Control	06 days	12 days		Control	06 Days	12 Days	
BSMR-736	47.61	46.03	44.84	Low	1.53	1.24	1.47	Medium
Renuka	50.34	42.24	47.32	Medium	1.61	1.06	1.22	Low
BDN-711	55.00	46.12	49.49	High	1.29	1.12	1.81	High
BDN-2	55.26	43.10	40.89	High	1.46	1.14	1.82	High
Vipula	49.00	45.59	46.45	Medium	1.72	1.17	1.42	Medium
Phule Rajeshwari	46.93	41.15	45.83	Medium	1.63	1.20	1.77	High
AKT-8811	50.73	44.06	45.01	Medium	1.41	1.23	1.75	Medium
Godawari	56.84	42.61	50.86	High	1.54	1.15	1.86	High
BSMR 853	51.73	39.37	48.54	High	1.50	1.24	1.78	Medium
BDN-716	52.46	44.06	49.48	High	1.69	1.31	1.84	High
BDN-708	46.71	34.25	41.03	Low	1.64	1.26	1.44	Medium
PKV-Tara	41.50	34.24	39.11	Low	1.28	1.16	1.62	Low

Comparative analysis of physiological characters during control and drought responses of chickpea genotypes

Previous studies demonstrated that expressing *ASR gene* during drought treatment enhanced drought tolerance in chickpea (*Cicer arietinum* L.) and was associated with increases in shoot and root growth, photosynthetic activities, and WUE plants exposed to drought stress (Supriya Sachdeva *et al.*, 2020). In the current study, physiological screening and qRT-PCR results demonstrated increased ASR gene expression under drought stress possibly enabled genotypes to perform better under stress. Conserved domain search, protein structure analysis, prediction and validation,

network analysis using Phyre2, Swiss-PDB viewer, ProSA and STRING analysis established the role of hypothetical ASR protein NP_001351739.1 in mediating drought responses. NP_001351739.1 might have enhanced the ASR gene activity as a transcription factor regulating drought stress tolerance in chickpea. This study could be useful in identification of new ASR genes that play a major role in drought tolerance and also develop functional markers for chickpea improvement.

In the current study, the analysis of physiological responses to water stress for the protein content (leaf), weight of fresh shoot, relative water content and shoot dry matter content

demonstrated that *ASR* gene expression in drought stress and in control condition neither improve not reduced in water

stress, which is helped to maintain studied physiological characters under water stress.

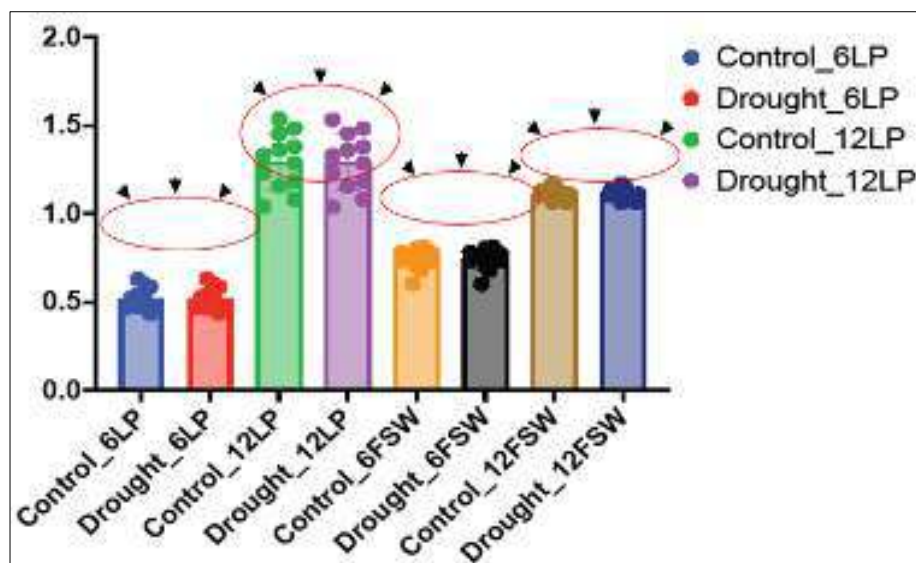


Fig 6: Comparison between physiological characters during control and drought responses of chickpea genotypes measured after different periods of stress (0, 6 and 12 days after drought stress treatment).

In the T-test analysis of physiological characters, it was found that did not found any significant difference between

group of six day and drought leaf protein responses of chickpea genotypes.

Table 4: T-test for comparison between group of six day and drought leaf protein responses of chickpea genotypes (Version: 1.0, Start HTML: 0000000105, End HTML: 0000005373, Start Fragment: 0000002160, End Fragment: 0000005327)

Table Analyzed	Data 1
Column B	Drought_6LP
vs.	vs.
Column A	Control_6LP
Paired t test	
P value	0.4584
P value summary	ns
Significantly different (P < 0.05)?	No
One- or two-tailed P value?	Two-tailed
t, df	t=0.7684, df=11
Number of pairs	12
How big is the difference?	
Mean of differences (B - A)	-0.0005000
SD of differences	0.002254
SEM of differences	0.0006507
95% confidence interval	-0.001932 to 0.0009322
R squared (partial eta squared)	0.05094
How effective was the pairing?	
Correlation coefficient (r)	0.9993
P value (one tailed)	<0.0001
P value summary	****
Was the pairing significantly effective?	Yes

Profiling for grouping of physiological characteristics of pigeon pea genotypes under drought stress condition

The analysis of physiological responses to water stress for the protein content (leaf), weight of fresh shoot, relative water content and shoot dry matter content demonstrated that *ASR* gene expression in drought stress and in control condition is highly non-significant. Identification and grouping of pigeon pea genotypes was based on protein

content (leaf) ($\mu\text{g/ml}$), weight of fresh shoot (gm), relative water content (%) and shoot dry matter content (gm). Total three scorable groups were recorded among all physiological responses and depicted monomorphism or common range in all genotypes. Cluster analysis via UPGMA procedure exposed four major groups observed at 100% homology or similarity within the group.

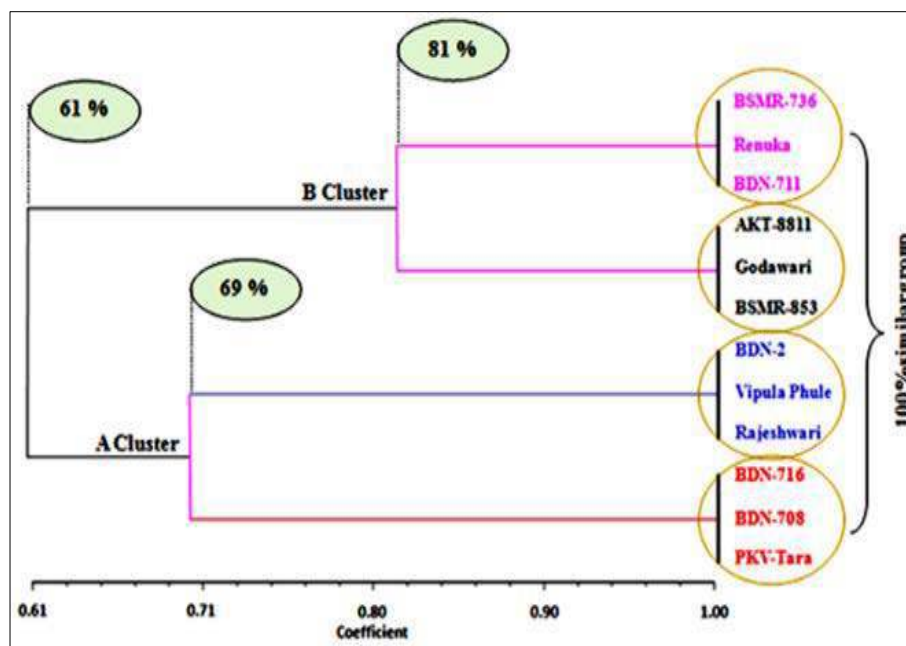


Fig 7: Dendrogram for 12 pigeon pea genotypes based on NTSYS-pc UPGMA clustering method with similarity from physiological traits analysis

Similarity coefficient ranged from 0.61 to 1.00 with an elucidian distance of 0.39. (Fig 7). Highest genetic coefficient similarity (0.61) was observed between first main A cluster and second main B cluster. Further, the dendrogram constructed from the pooled physiological data clearly showed, two major clusters namely cluster A and B. Six genotypes each was found on cluster A and cluster B respectively. In cluster A, and cluster B genotypes were found 100% similarity with each other (Table 5 and Table 6). Two main clusters had 61 per cent similarity found within 12 genotypes at physiological level.

Whereas similarity NTSYS-pc method for physiological traits data was divided into two major clusters, and 100%

similarity are shared as in four groups, each cluster contains three genotypes cluster A and B contains six genotypes respectively. NTSYS-pc UPGMA clustering method with similarity from physiological traits analysis revealed not much amount of physiological diversity, across all pigeon pea genotypes. This statement was well supported by the analysis of RAPD data. While the physiological traits profiles across all pigeon pea genotypes in this study and belonging to two different reading groups (low and high) and were only able to show polymorphism with a 12 genotypes with an elucidian distance of 0.39 and indicating not much amount of variation between the pigeon pea genotypes (Table 6).

Table 5: The list pigeon pea genotypes of cluster analysis based on physiological traits profiles analysis with name and origin

Name of Cluster	Code of pigeon pea genotypes	Origin
Main Cluster A 06 pigeon pea genotypes		A.R.S., Badnapur
Main Cluster B 06 pigeon pea genotypes		A.R.S., Badnapur
1.	Cluster A	
a.	Cluster A ₁ (03 pigeon pea genotypes)	
b.	Cluster A ₂ (03 pigeon pea genotypes)	
Sub-Cluster A ₁	PKV-Tara, BDN-708 and BDN-716	100% similar to each other
Sub-Cluster A ₂	Rajeshwari, Vipula Phule and BDN-2	100% similar to each other
2.	Cluster B	
a.	Cluster B ₁ (03 pigeon pea genotypes)	
b.	Cluster B ₂ (03 pigeon pea genotypes)	
Sub-Cluster B ₁	BSMR-853, Godawari and AKT-8811	100% similar to each other
Sub-Cluster B ₂	BDN-711, Renuka and BSMR-736	100% similar to each other

Genetic diversity analysis using RAPD markers Similarity coefficient

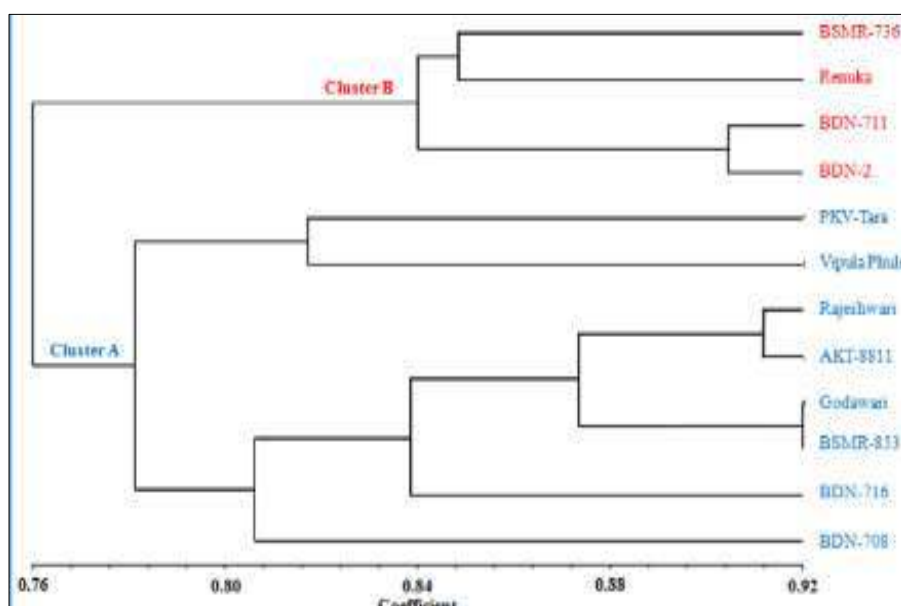
To assess the set of 12 pigeon pea genotypes at molecular level by using RAPD marker. The observations of RAPD primers were scored for the presence (1) or absent (0) of bands on the protein profiles and the data was then subjected to generate similarity matrix using the program similarity for qualitative data (SIMQUAL) dice coefficient. Cluster analysis was carried out by the UPGMA method and

dendrogram thus generated using SAHN subroutine of NTSYS-pc is summarized in Table 7 and fig 8.

A perusal of dendrogram Figure 8 indicated that the similarity coefficient ranged from 0.76 to 0.92 among 12 pigeon pea genotypes based on RAPD markers. The diversity in DNA level was analyzed with an elucidian distance of 0.16 indicating that pigeon pea genotypes did not show much variation at DNA level.

Table 6: Similarity matrix analysis for physiological traits data coefficient study

	BSMR-736	Renuka	BDN-711	BDN-02	Vipula	Phule Rajeshwari	AKT-8811	Godawari	BSMR-853	BDN-716	BDN-708	PKV-Tara
BSMR-736	1.00											
Renuka	0.84	1.00										
BDN-711	0.82	0.79	1.00									
BDN-02	0.85	0.86	0.90	1.00								
Vipula	0.78	0.75	0.72	0.77	1.00							
Phule Rajeshwari	0.80	0.76	0.73	0.74	0.81	1.00						
AKT-8811	0.74	0.75	0.74	0.79	0.76	0.84	1.00					
Godawari	0.72	0.78	0.75	0.82	0.79	0.77	0.90	1.00				
BSMR-853	0.70	0.72	0.72	0.76	0.70	0.77	0.86	0.82	1.00			
BDN-716	0.76	0.78	0.76	0.84	0.78	0.82	0.91	0.88	0.91	1.00		
BDN-708	0.81	0.79	0.68	0.75	0.78	0.82	0.85	0.81	0.80	0.86	1.00	
PKV-Tara	0.71	0.67	0.71	0.73	0.69	0.75	0.82	0.77	0.80	0.80	0.80	1.00
Maximum	0.85	0.86	0.90	0.84	0.81	0.84	0.91	0.88	0.91	0.86	0.85	0.82
Minimum	0.71	0.67	0.68	0.73	0.69	0.75	0.82	0.77	0.80	0.80	0.68	0.69

**Fig 8:** Dendrogram for 12 pigeon pea genotypes based on NTSYS-pc UPGMA clustering method with similarity from RAPD markers analysis

The twelve pigeon pea genotypes were grouped into two major clusters A and B. The cluster A contains 08 pigeon pea genotypes while cluster B has only four genotypes and shared 92% dissimilarity or minimum 76% similarity. The cluster A was sub grouped as A₁, and A₂ consisted of 01 and 05 genotypes and shared 81% similarity among them (Figure 8).

The subgroup A₁ consisted of 01 genotype (BDN-708) and shared 81% similarity. The subgroup A₂ was further subdivided into A₂ (a) and A₂ (b) and consisted of 01 and 04 genotypes, shared 84% similarity.

Whereas subgroup A₂ (b) was subdivided into two sub-sub clusters A₂ (a₁) and A₂ (a₂) consisted of 02, and 02 genotypes, shared 87% similarity among 04 genotypes. While A₂ (a₁) sub-sub clusters having BSMR-853 and Godawari genotypes are 100% similar to each other's.

One group was identified within which all the pigeon pea genotypes share 100% similarity (BSMR-853 and Godawari) for molecular (RAPD) data, and also one group was also identified contained single line with 100% dissimilarity (BDN-708).

Similarity matrix analysis for molecular (RAPD) coefficient study

Similarity coefficient ranged from 0.76 to 0.92 with an elucidian distance of 0.16. (Figure 8). Highest genetic coefficient similarity (0.92) was observed within 12 genotypes. NTSYS-pc UPGMA clustering method with similarity from molecular markers analysis revealed not much amount of genetic diversity, across all pigeon pea genotypes. This statement was well supported by the analysis of RAPD data. While the polymorphism within a 12 genotypes indicating not much amount of genetic variation between the pigeon pea genotypes (Table 7).

Estimation of the level of allelic polymorphism and monomorphism in a set of pigeon pea genotypes using RAPD marker

Genotyping of 12 pigeon pea genotypes were done by 36 RAPD primers generated differential banding patterns among different genotypes. All RAPD primers were able to generate 1775 amplicons with an average 49.30 amplicons per primer (Table 8). Out of total 1775 amplicons 287 were detected polymorphism which showed 25.77% polymorphism among 12 pigeon pea with 9.61 average numbers of polymorphic bands. The numbers of amplified fragments for primers ranged from 01 (OPN-16) to 05 (OPA-16) whereas the band size was ranged from 100bp to

750bp. OPA-16 primer was the most informative primer as it generated the highest number of polymorphic amplicons i.e. 21 polymorphic amplicons while A20 and OPN-04 stood least informative by generated less numbers of polymorphic amplicons. The lowest numbers of amplified fragments were generated by primer OPN-06 and OPA-02 (Table 8).

Genotyping of 12 pigeon pea genotypes were done by 36 RAPD primers generated differential banding patterns among different genotypes. All RAPD primers were able to generate 1775 amplicons with an average 49.30 amplicons per primer (Table 9).

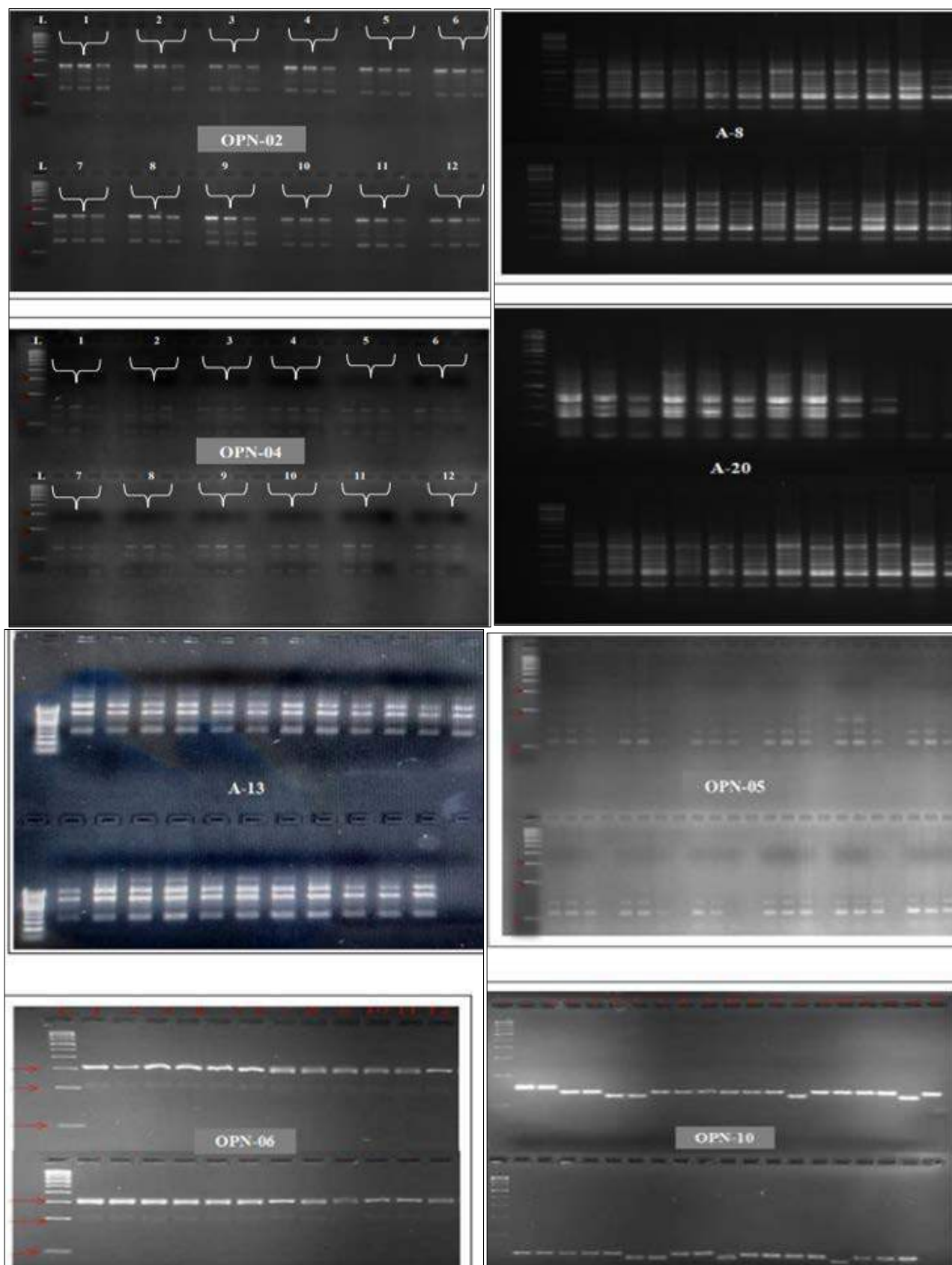


Fig 9: Genotyping of 12 pigeon pea genotypes using RAPD markers

Out of total 1775 amplicons 1429 were detected monomorphism which showed 77.07% monomorphism among 12 pigeon pea genotypes with 39.69 average numbers of monomorphic bands. The numbers of amplified fragments for primers ranged from 01 (OPN-16) to 05 (OPA-16) whereas the band size was ranged from 100bp to 750bp. OPN-06 and OPA-02 primer was the less

informative primer as it generated the lowest number of polymorphic amplicons i.e. 00 polymorphic amplicons while A20 and OPN-04 stood least informative by generated less numbers of polymorphic amplicons. OPA-16 primer was the most informative primer as it generated the highest number of polymorphic amplicons i.e. 21 polymorphic amplicons (Table 9).

Table 7 Similarity matrix analysis for molecular markers (RAPD) data coefficient study

	BSMR-736	Renuka	BDN-711	BDN-02	Vipula	Phule Rajeshwari	AKT-8811	Godawari	BSMR-853	BDN-716	BDN-708	PKV-Tara
BSMR-736	1.00											
Renuka	0.84	1.00										
BDN-711	0.82	0.79	1.00									
BDN-02	0.85	0.86	0.90	1.00								
Vipula	0.78	0.75	0.72	0.77	1.00							
Phule Rajeshwari	0.80	0.76	0.73	0.74	0.81	1.00						
AKT-8811	0.74	0.75	0.74	0.79	0.76	0.84	1.00					
Godawari	0.72	0.78	0.75	0.82	0.79	0.77	0.90	1.00				
BSMR-853	0.70	0.72	0.72	0.76	0.70	0.77	0.86	0.82	1.00			
BDN-716	0.76	0.78	0.76	0.84	0.78	0.82	0.91	0.88	0.91	1.00		
BDN-708	0.81	0.79	0.68	0.85	0.78	0.82	0.85	0.81	0.80	0.86	1.00	
PKV-Tara	0.71	0.67	0.71	0.73	0.69	0.75	0.82	0.77	0.80	0.80	0.80	1.00
Maximum	0.84	0.86	0.90	0.85	0.81	0.84	0.91	0.88	0.91	0.86	0.86	0.86
Minimum	0.70	0.72	0.68	0.73	0.69	0.75	0.82	0.77	0.80	0.80	0.68	0.68

Table 8: Polymorphic amplicons generated by RAPD markers.

Sr. No.	Primers	Total Number of Bands	Average Band/ Primer	Polymorphic Band	Percent Polymorphism (%)
1	A8	109	9.08	11	10.09
2	A13	46	3.83	14	30.43
3	A20	105	8.75	03	02.85
4	D3	51	4.25	09	17.64
5	B7	15	1.25	09	60.00
6	T14	36	3.00	00	00.00
7	OPN-02	37	3.08	11	29.72
8	OPN-04	46	3.83	02	04.34
9	OPN-05	36	3.00	00	00.00
10	OPN-06	36	3.00	00	00.00
11	OPN-10	23	1.91	11	47.82
12	OPN-11	29	2.41	07	24.13
13	OPN-12	41	3.41	07	17.07
14	OPN-15	102	8.50	17	16.66
15	OPN-16	87	7.25	21	24.13
16	OPN-17	30	2.50	06	20.00
17	OPA-01	15	1.25	09	60.00
18	OPA-02	24	2.00	00	00.00
19	OPA-03	35	2.91	13	37.14
20	OPA-04	86	7.16	10	11.62
21	OPA-05	34	2.83	14	41.17
22	OPA-06	54	4.50	18	33.33
23	OPA-07	49	4.08	11	22.44
24	OPA-08	27	2.25	09	33.33
25	OPA-09	54	4.50	06	11.11
26	OPA-10	90	7.50	18	20.00
27	OPA-11	32	2.60	04	12.50
28	OPA-12	43	3.50	05	11.62
29	OPA-13	50	4.16	10	20.00
30	OPA-14	52	4.33	08	15.38
31	OPA-15	83	6.91	13	15.66
32	OPA-16	87	7.25	21	24.13
33	OPA-17	30	2.50	06	20.00
34	OPA-18	34	2.83	14	41.17
35	OPA-19	37	3.08	11	29.72
36	OPA-20	30	2.50	18	60.00

Note: Average no. of amplicons per primer = 49.30

Average no. of polymorphic amplicons per primer = 9.61

Total percent polymorphism by RAPD marker = 25.77%

Table 9: Monomorphic amplicons generated by RAPD markers.

Sr. No.	Primers	Total Number of Bands	Average Band/ Primer	Monomorphic Band	Percent Monomorphism (%)
1	A8	109	9.08	98	89.90%
2	A13	46	3.83	32	69.56%
3	A20	105	8.75	102	97.14%
4	D3	51	4.25	42	82.35%
5	B7	15	1.25	06	40%
6	T14	36	3.00	36	100%
7	OPN-02	37	3.08	26	70.27%
8	OPN-04	46	3.83	44	95.65%
9	OPN-05	36	3.00	36	100%
10	OPN-06	36	0.00	36	100%
11	OPN-10	23	1.91	12	52.17%
12	OPN-11	29	2.41	22	75.86%
13	OPN-12	41	3.41	34	82.92%
14	OPN-15	102	8.50	85	83.33%
15	OPN-16	87	7.25	66	75.86%
16	OPN-17	30	2.5	24	80%
17	OPA-01	15	1.25	6	40%
18	OPA-02	24	2.00	24	100%
19	OPA-03	35	2.91	22	62.85%
20	OPA-04	86	7.16	76	88.37%
21	OPA-05	34	2.83	20	58.82%
22	OPA-06	54	4.50	36	66.66%
23	OPA-07	49	4.08	38	77.55%
24	OPA-08	27	2.25	18	66.66%
25	OPA-09	54	4.50	48	88.88%
26	OPA-10	90	7.50	72	80%
27	OPA-11	32	2.60	28	87.5%
28	OPA-12	43	3.50	38	88.37%
29	OPA-13	50	4.16	40	80%
30	OPA-14	52	4.33	44	84.61%
31	OPA-15	83	6.91	70	84.33%
32	OPA-16	87	7.25	66	75.86%
33	OPA-17	30	2.50	24	80%
34	OPA-18	34	2.83	20	58.82%
35	OPA-19	37	3.08	26	70.27%
36	OPA-20	30	2.50	12	40%

Note: Average no. of amplicons per primer = 49.30

Average no. of polymorphic amplicons per primer = 30.69

Total percent polymorphism by RAPD marker = 77.07%

Identification and characterization of ASR gene in pigeon pea genotypes

The genomic DNA of 12 pigeon pea genotypes was extracted from young leaves using the CTAB method (Doyle J. (1991) ^[5]. Purified DNA was used for PCR amplification using ASR gene specific

Forward 5'-GGGAACCTAATCCTTTCCAAACA-3' and Reverse 5'-CTGCAGCACCTAACTCACCA-3' primer custom was synthesized and PCR was carried out in the VDCOAB, Latur, Plant Biotechnology Laboratory using a thermal cycler (Fig 10).

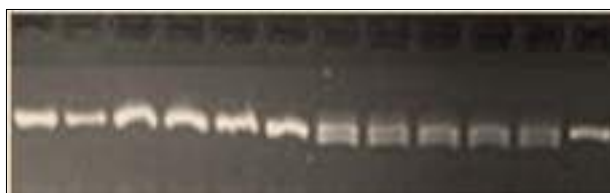


Fig 10: Genomic DNA isolation of 12 pigeon pea genotypes by CTAB method

The PCR master mix comprised of 20ng of the template DNA, 1.6µl of 10X Tris borate-ethylenediamine-tetra acetic acid (TBE), 1µl of 10mM dNTP mix, 1µl each of 5µM

forward and reverse primer and 0.3 µl of 3U *Taq Polymerase*. The PCR cycling reaction consisted of three steps, starting with initial denaturation (90 °C for 3 min) followed by 38 cycles of denaturation (94 °C for 20 sec), annealing (55 °C for 50 sec) and elongation (72 °C for 50 sec) followed by final elongation (72 °C for 7 mins).

The PCR amplicons were visualized on a 3% 1X TBE buffer using 100 bp DNA ladder. The gel image was documented using UV light gel documentation system.

Molecular confirmation of ASR gene in pigeon pea genotypes

The gene specific primer which was synthesized and PCR were not found to be amplification in all 12 pigeon pea genotypes. PCR of isolated DNA from 12 genotypes was performed using gene specific primer and programmed used for confirmation of amplification of ASR gene in all genotypes.

Real-time gene expression analysis by semi-quantitative and quantitative RT-PCR requires a set of gene-specific primers which should have the ability to amplify the gene of interest specifically.

In the present study, standardized certain parameters for primer design using the freely available Primer3 software.



Fig 11: Negative amplification during RT-PCR of all 12 genotypes for ASR gene of interest

The primers designed for defense genes such as ICS (Isochorismate synthase), CCoAOMT (caffeoyl CoA *O*-methyltransferase), C4H (cinnamate 4-hydroxylase), and G-alpha in pea. In the present study discussed the way of sequence retrieval, when the sequence is not reported in the organism of interest. The designed forward and reverse primers were evaluated using cDNA prepared from mRNA isolated from the pigeon pea leaves. By analysing the results, it have found that designed primers are perfect but not binding with the target and giving single sharp band on a DNA electrophoresis gel. It can be concluded that the parameters used for primer designing by Primer3 play a critical role in the experimental results and parameters defined in the present study resulting in a negative amplification during PCR of all 12 genotypes.

Confirmation and phylogenetic radial tree analysis for synthesized ASR gene marker for pigeon pea

The ASR gene homologues were synthesized from *Medicago* gene sequence available at NCBI EST database (DbEST-<http://www.Ncbi.nlm.nih.gov/dbEST/>) (Philippe R. *et al.*, 2010). Sequences of the ASR genes ranged from 680bp to 700bp nucleotides. Conserved region was not observed in all the pigeon pea genotypes (Fig 11). BLASTn results confirmed 99.32% similarity with pigeon pea putative ABA/WDS induced protein (LOC101493413), mRNA with E value of 0.00. In the previous study reveals that the presence of ASR gene sequence ranged from 680bp to 700 bp in chickpea genotypes (Supriya Sachdeva *et al.*, 2020) Figure 12.

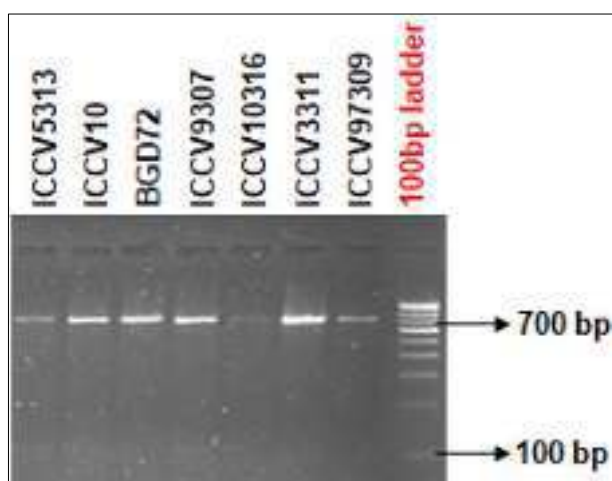


Fig 12: PCR Amplification of seven chickpea genotypes using ASR gene specific marker. PCR amplification of seven chickpea genotypes viz., ICCV97309, ICCV3311, ICCV10316, ICCV9307, BGD72, ICCV10, and ICCV5313 was done using ASR gene specific primer and revealed a single amplicon ranging from 680-700bp; Marker-100 bp Bangalore Genei DNA ladder.

In the BLASTn comparative analysis, first eight sequences depicting 100 percent similar results with the synthesized ASR gene sequence. Remaining two sequences are 95 percent similar results with the synthesized ASR gene sequence. In the present study, all the 12 pigeon pea genotypes don't show the amplification of ASR gene.

Conclusions

1. The physiological characters studied can be utilized for identification and characterization of pigeon of in relation to ASR gene testing.
2. These characters are also helpful towards identification and characterization of pigeon of types in relation to occurrence of ASR gene.
3. All the physiological characters results included in this study are depicted in not in much amount of variation identified in between 12 pigeon of types in relation to expression of ASR gene. The marginal difference which is considered as negligible difference shows that the absence of ASR gene in all 12 pigeon of types.
4. With reference to the non-significant results of physiological characters study the RAPD markers were utilized for the characterization of 12 pigeon of types at DNA level, which is not influenced by the environment. These molecular markers are helpful in assessing genetic purity at a shortest time.
5. A perusal of dendrogram indicated that the similarity coefficient ranged from 0.76 to 0.92 among 12 pigeon pea genotypes based on RAPD markers. The diversity in DNA level was analyzed with anelucidian distance of 0.16 indicating that pigeon pea genotypes did not show much variation at DNA level. All the genotypes were divided into only two main clusters at 76 percent similarity.
6. All RAPD primers were able to generate 1775 amplicons with an average 49.30 amplicons per primer. Out of total 1775 amplicons polymorphism detected only 25.77% polymorphism with 9.61 average numbers of polymorphic bands.
7. Whereas the band size was ranged from 100bp to 750bp included both polymorphic and monomorphic bands. The genotyping of 12 pigeon pea genotypes were done by 36 RAPD primers generated differential polymorphic and monomorphic banding patterns among different genotypes.
8. The designed primer (s) are perfect but not binding with the target and giving single sharp band on a DNA electrophoresis gel. It can be concluded that the parameters used for primer designing by Primer3 play a critical role in the experimental results and parameters defined in the present study resulting in a negative amplification during PCR of all 12 genotypes.
9. In the BLASTn comparative analysis with designed primers, first eight sequences depicting 100 percent similar results with the synthesized ASR gene sequence. Remaining two sequences are 95 percent similar results with the synthesized ASR gene sequence. In the present study, all the 12 pigeon pea genotypes don't show the amplification of ASR gene.
10. Therefore, it was concluded that the 12 pigeon pea genotypes collected from ARS, Badnapur don't show the amplification of ASR gene. According to the comparative analysis results of all genotypes belongs to drought susceptible.

References

1. Abrol DP, Shankar U. Role of Pollination in Pulses. *Adv Pollen Spore Res.* 2015;33:101–3.
2. Chaimala A, Jogloy S, Vorasoot N, Toomsan B, Jongrunklang N, Kesmla T, *et al.* Responses of total biomass, shoot dry weight, yield and yield components of Jerusalem artichoke (*Helianthus tuberosus* L.) varieties under different terminal drought duration. *Agriculture*; c2020 .p. 1-15.
3. Basu TK, Bandyopadhyay SR. Productivity of rabi pigeonpea (*Cajanus cajan* L. Millsp.) as influenced by scheduling of irrigation. *J Crop Weed.* 2009;5(2):90–1.
4. Choudhary AK, Kumar S, Patil BS, Bhat JS, Sharma M. Narrowing yield gaps through genetic improvement for Fusarium wilt resistance in three pulse crops of the semiarid tropics. *Sabroa J Breed Genet.* 2013;45:341–70.
5. Doyle J. DNA Protocols for Plants. In: *Molecular Techniques in Taxonomy*; c1991. p. 28.
6. Dure L, Crouch M, Harada J, Ho THD, Mundy J, Quatrano R, *et al.* Common amino acid sequence domains among the LEA proteins of higher plants. *Plant Mol Biol.* 1989;12:475–86.
7. Edwards D, Selden PA. The development of early terrestrial ecosystems. *Bot J Scotl.* 1992;42:337–66.
8. Finkelstein RR, Gampala SS, Rock CD. Absciscic acid signaling in seeds and seedlings. *Plant Cell.* 2002;14(Suppl)–45.
9. Frankel N, Carrari F, Hasso E, Iusem ND. Evolutionary history of the Asr gene family. *Gene.* 2006;378:74–83.
10. Hamdi K, Brini F, Kharrat N, Masmoudi K, Yakoubi I. Absciscic Acid, Stress, and Ripening (TtASR1) Gene as a Functional Marker for Salt Tolerance in Durum Wheat. *Biomed Res Int.* 2020;2020:7876357. DOI:10.1155/2020/7876357.
11. Henry IM, Carpentier SC, Pampurova S, Van Hoylandt A, Panis B, Swennen R, *et al.* Structure and regulation of the Asr gene family in banana. *Planta.* 2011;234:785–98.
12. Iusem ND, Bartholomew DM, Hitz WD, Scolnik PA. Tomato (*Lycopersicon esculentum*) transcript induced by water deficit and ripening. *Plant Physiol.* 1993;102:1353–4.
13. Jin SY, Kim JY, Kim DY, Seo YW. A novel wheat ASR gene, TaASR2D, enhances drought tolerance in *Brachypodium distachyon*. *Plant Physiol Biochem.* 2021;159:400–14.
14. Kopecka R, Kameniarova M, Cerny M, Brzobohaty B, Novak J. Abiotic Stress in Crop Production. *Int J Mol Sci.* 2023;24(7):6603. DOI:10.3390/ijms24076603.
15. Leung J, Giraudat J. Absciscic acid signal transduction. *Annu Rev Plant Physiol Plant Mol Biol.* 1998;19:199–222.
16. Oda M, Tsuji K. Monitoring Fresh Weight of Leaf Lettuce. 2. Effects of light, air temperature, relative humidity and wind velocity. *JARQ.* 1992;26:19–25.
17. Mathlouthi N, Yacoubi I, Gadaleta A, Hamdi K, Giancaspro A. Absciscic Acid Stress-Ripening Genes involved in plant response to high salinity and water deficit in durum and common wheat. *Front Plant Sci.* 2022;13:10.
18. Odeny DA. The potential of pigeon pea (*Cajanus cajan* (L.) Millsp.) in Africa. *Nat Resour Forum.* 2007;31:297–305.
19. Philippe R, Courtois B, McNally KL, Mournet P, El-Malki R, Le Paslier MC. Structure, allelic diversity and selection of Asr genes, candidates for drought tolerance, in *Oryza sativa* L. and wild relatives. *Theor Appl Genet.* 2010;121(4):769–87. DOI:10.1007/s00122-010-1348.
20. Qiao G, Wen XP, Yu LF, Ji XB. The enhancement of drought tolerance for pigeon pea inoculated by arbuscular mycorrhizae fungi. *Plant Soil Environ.* 2011;57:541–6. DOI:10.17221/116/2011-PSE.
21. Rabbani MA, Maruyama K, Abe H, Khan MA, Katsura K, Ito Y, *et al.* Monitoring expression profiles of rice genes under cold, drought, and high-salinity stresses and abscisic acid application using cDNA microarray and RNA gel-blot analyses. *Plant Physiol.* 2003;133:1755–67.
22. Sachdeva S, Bharadwaj C, Singh RK, Jain PK, Patil BS, Roorkiwal M. Characterization of ASR gene and its role in drought tolerance in chickpea (*Cicer arietinum* L.). *PLoS One.* 2020, 15(7).
23. Shinozaki K, Yamaguchi-Shinozaki K. Gene networks involved in drought stress response and tolerance. *J Exp Bot.* 2007;58(2):221–7. DOI:10.1093/jxb/erl164.
24. Silhavy D, Hutvagner G, Barta E, Banfalvi Z. Isolation and characterization of a water-stress-inducible cDNA clone from *Solanum chacoense*. *Plant Mol Biol.* 1995;27:587–95.
25. Sinha P, Singh VK, Saxena RK, Khan AW, Abbai R, Chitikineni A, *et al.* Superior haplotypes for haplotype-based breeding for drought tolerance in pigeon pea (*Cajanus cajan* L.). *Plant Biotechnol J.* 2020:1–9.
26. Song Z, Dong B, Yang Q, Niu L, Li H, Cao H, *et al.* Screening of CBL genes in pigeon pea with focus on the functional analysis of CBL4 in abiotic stress tolerance and flavonoid biosynthesis. *Environ Exp Bot.* 2020;177:10410. DOI:10.1016/j.envexpbot.2020.10410.