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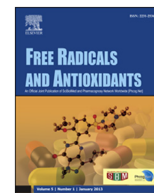

Original article

Studies on antioxidants and peroxidase isoenzymes in seedlings of twelve cultivars with four different durations of flowering time in pigeon pea (*Cajanus cajan* (L.) Millspaugh)

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ABSTRACT

Objective: The present study is an attempt to understand the role of enzymatic and non-enzymatic antioxidants as well as peroxidase isoenzymes in order to differentiate the four flowering durations of pigeon pea cultivars.
Methods: The assay of superoxide dismutase, catalase, peroxidase, ascorbate oxidase, ascorbic acid, reduced glutathione, lipid peroxidation, protein and phenolic content was done for the evaluation of antioxidant activity. For peroxidase isozymes assay, native-PAGE was carried out with 10% acrylamide gels at 4 °C without SDS and β-mercaptoethanol.
Results: Among all the cultivars ICP 15599, which is an extra-early flowering variety showed highest levels of both enzymatic and non-enzymatic antioxidants and least lipid peroxidation. Isoenzyme marker analysis of POD showed six common isoforms in all the 12 varieties and an additional isoform in ICP 15599.
Conclusion: Among four different flowering durations extra-early group showed highest activity of enzymatic and non-enzymatic antioxidants, whereas lipid peroxidation was least. The peroxidase isozyme analysis showed there is no specific isoform for flowering time identification, but however there is one marker for extra-early cultivar (ICP 15599).
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1. Introduction

Germination and flowering are both fundamental developmental transitions that require precise environmental sensing and responses to multiple seasonal cues. The combination of these two phenological traits determines the overall life cycle and generation time of many plants.[1] Because both transitions respond to similar seasonal cues, it is logical to hypothesize that genetic pathways of these two life-history transitions share common elements. Pigeon pea (*Cajanus cajan* (L.) Millsp., Family – Fabaceae), is one of the major pulse crops of the tropics and sub-tropics and is mostly intercropped with cereals. It is a short-day plant,[2] and so the onset of flowering is delayed in longer rather than shorter days.[3] Based on time of flowering, pigeon pea genotypes have been classified into four major maturity groups: the extra-early, early, medium and late genotypes.

Seed germination and flowering are complex processes requiring a multidisciplinary approach in analysis.[4] The sequence of the metabolic pattern that occurs during flowering involves the activation of specific enzymes at the appropriate times and regulation of their activity. Accumulation of active oxygen species (AOS), during seed imbibition, leads to germination.[5] Appearance of AOS in the plant cells is generally linked with the involvement of free radical in plant development, as well as its interaction with the environment.[6,7] On the other hand, some of them, such as H_2O_2 and O_2^- are proposed to have a signaling role in the cell during stress action.[8,9] A regulated balance between oxygen radical production and destruction is required if metabolic efficiency and function are to be maintained in both optimal and stress conditions. In plant cells the detoxifying enzymes (peroxidase, catalase, superoxide

Abbreviations: AOS, active oxygen species; SOD, superoxide dismutase; POD, peroxidase; CAT, catalase; ASO, ascorbate oxidase; ASH, ascorbic acid; GSH, reduced glutathione; MDA, malondialdehyde; PAGE, polyacrylamide gel-electrophoresis; SDS, sodium dodecyl sulfate.

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dismutase and ascorbate oxidase) along with reduced glutathione and ascorbic acid have an important role throughout plant ontogeny, from seed germination[5] to growth and development.[10,11] Malondialdehyde (MDA) is considered sensitive marker commonly used for assessing membrane lipid peroxidation.[12]

The increased economic importance of the crop has stimulated the development of new cultivars. Therefore, identification of varieties became very important in seed certification and crop breeding programs to screen different plant genotypes. At present, morphological features are commonly used to identify crop cultivars. For some plant species, identification based on plant and seed morphology has been unreliable, because morphological characters can be affected by environmental conditions.[13] Moreover, a cultivar also must be judged by an individual who possesses a thorough knowledge of the cultivar at the precise time. For morphological characterization, the plant must be grown to flowering or fruiting stage, which is space and time consuming. Therefore, it is desirable if a cultivar identification system could be developed, based on biochemical techniques.[14]

Hence the present study is an attempt to understand the role of enzymatic and non-enzymatic antioxidants as well as peroxidase isoenzymes in order to differentiate the four flowering durations of pigeon pea.

2. Materials and methods

2.1. Chemicals

Bovine serum albumin (BSA), 2,6-dichloro phenol indophenol, reduced glutathione (GSH), thiobarbituric acid (TBA), acrylamide, bis acrylamide, sodium dodecyl sulfate (SDS), ammonium persulphate (APS), β-mercaptoethanol, coomassie brilliant blue R 250 were obtained from Himedia Laboratories Pvt. Limited, Mumbai, India. Gallic acid, mercuric chloride, hydroxylamine hydrochloride, sodium carbonate, potassium permanganate, oxalic acid, meta phosphoric acid, 5, 5′-dithiobis (2-nitrobenzoic acid) (DTNB) were obtained from Qualigens, Mumbai, India. Nitroblue tetrazolium (NBT) were obtained from Merck Limited, Mumbai, India.

2.2. Plant material

Twelve pigeon pea cultivars belonging to four flowering durations namely extra-early (ICP 7220, ICP 15598, ICP 15599), early (ICP 26, ICP 28, ICP 1124), medium (ICP 472, ICP 3437, ICP 7118), late (ICP 1342, ICP 1406, ICP 1433) were used in the present study. The seeds were obtained from ICRISAT, Patancheru, Andhra Pradesh, India. Seeds of uniform size were selected and soaked in distilled water for 2 h and were surface sterilized with 0.1% mercuric chloride for 2 min. The seeds were then washed thoroughly with sterile distilled water. The washed seeds were then spread over petri dishes lined with two-layered moistened filter paper. The seeds of these cultivars were allowed to germinate at 30 ± 2 °C for 6 days under a photoperiod of 16 h day light and 8 h dark. Then the 6 days old seedlings were collected and used for experimental analysis. Radicle emergence of 2 mm was considered as germination.[15] Germination percentage was determined for each cultivar. The experiment was repeated thrice with sample size of 20 seeds.

2.3. Preparation of extract

About 1 g seedlings of each cultivar were homogenized separately with 7.5 mL of pre-chilled phosphate buffer (0.1 M, pH 7.6), containing 0.1 mM EDTA in chilled mortar and pestle. The homogenate was squeezed through double layered cheese cloth and centrifuged (Sorvall Instrument RC5C, Rotor SS-34) at 16,000 rpm for 15 min at 4 °C. The supernatant was used for the assay of SOD, CAT, POD, and ASO also for non-enzymatic antioxidants like total phenols, reduced glutathione, ascorbic acid, and proteins. The assay of SOD was carried by the method of Beauchamp and Fridovich based on the reduction of Nitroblue tetrazolium (NBT).[16] CAT activity was assayed by the titrimetric method described by Radhakissnan and Sarma.[17] Peroxidase (POD) activity was assayed spectrophotometrically (Shimadzu UV-265, UV–visible recording spectrophotometer) with O-dianisidine as hydrogen donor.[18,19] Assay of ASO activity was carried out according to the procedure of Vines and Oberbacher.[20] The determination of ASH was carried out by the procedure given by Sadasivam and Balasubramanian.[21] The GSH content was determined by the Boyne and Ellman method.[22] Lipid peroxidation (LPO) of the plant extract was determined by estimating the MDA content following the method of Heath and Packer with slight modification.[23] Total protein was estimated by the method of Lowry et al[24] with Bovine Serum Albumin as standard. The total phenolic content was determined spectrophotometrically by the method described by Sadasivam and Manickam.[19] For POD isozymes assay, native-PAGE was carried out by a modified method of Davis[25] with a 10% acrylamide gels at 4 °C without SDS and β-mercaptoethanol.

2.4. Statistical analysis

Each experiment was repeated three times. Analysis of variance was conducted using one-way ANOVA test using SPSS 9.01 for Microsoft Windows and mean separations were carried out using Duncan's Multiple Range Test (DMRT). Statistical significance was determined at 5% ($P < 0.05$) level.

3. Results

3.1. Germination

Germination percentage of the 12 cultivars (four different durations of flowering time) of pigeon pea was shown in Table 1. All cultivars showed germination percentage of above 80% except ICP 15598 and ICP 15599, which showed 78.33% and 65% respectively [Table 1].

3.2. Enzymatic antioxidants

Duncan grouping showed that there is significant difference between four different groups in POD, CAT and ASO activities,

Table 1
Germination percentage of 12 pigeon pea cultivars.

Duration	Type of cultivar	Germination percentage (%)[C]
Extra-early	ICP 7220	90.0 ± 5.0
	ICP 15598	78.33 ± 5.77
	ICP 15599	65.0 ± 5.0
Early	ICP 26	90.0 ± 0.0
	ICP 28	100.0 ± 0.0
	ICP 1124	100.0 ± 0.0
Medium	ICP 472	95.0 ± 5.0
	ICP 3437	100.0 ± 0.0
	ICP 7118	91.67 ± 2.89
Late	ICP 1342	95.0 ± 5.0
	ICP 1406	76.67 ± 2.89
	ICP 1433	86.67 ± 2.89
		$F = 26.69$[B]

Values represent the mean ± standard deviation of three independent experiments.
[B] Not significant at 5% level.
[C] The values represent the mean ± standard deviation of three independent experiments.

Table 2
Activities of antioxidant enzymes (POD, CAT, SOD and ASO) in seedlings of four different durations of flowering time.

Duration	Levels of antioxidant enzymes			
	POD[C] units/mg protein/min (±SD)	CAT[C] units/mg protein/min (±SD)	SOD[C] units/mg protein/min (±SD)	ASO[C] units/mg protein/min (±SD)
Extra-early	2.26 + 0.38a	0.47 + 0.39a	17.96 + 9.92a	27.94 + 1.43a
Early	1.03 + 0.41c	0.25 + 0.02c	10.52 + 5.78a	14.24 + 1.78c
Medium	1.69 + 0.09b	0.39 + 0.03b	15.41 + 9.04a	19.96 + 0.44b
Late	1.43 + 0.04b	0.36 + 0.02b	13.64 + 7.98a	15.49 + 1.18c
	$F = 28.44$[A]	$F = 67.59$[A]	$F = 1.26$[B]	$F = 202.19$[A]

[A] 5% level of significance ($p < 0.05$).
[B] Not significant.
[C] The values represent the mean ± standard deviation of three independent experiments. Means within a column followed by the same letter are not significantly different from each other at $p = 0.05$ according to Duncan's multiple range test (DMRT).

where POD varies from 2.26 units/mg protein to 1.03 units/mg protein, CAT activity varies from 0.47 units/mg protein to 0.25 units/mg protein and ASO activity varies from 27.94 units/mg protein to 14.24 units/mg protein [Table 2].

The three cultivars from each group also showed significant difference in POD, CAT and ASO activities, where POD varies from 2.73 units/mg protein to 0.50 units/mg protein, CAT activity varies from 0.50 units/mg protein to 0.22 units/mg protein and ASO activity varies from 29.44 units/mg protein to 12.87 units/mg protein [Table 3]. But there is no significant difference observed in SOD levels both within each group and also between groups.

3.3. Non-enzymatic antioxidants

Duncan grouping showed that there is significant difference in ASH and GSH content between four groups, where ASH levels varies from 1974.11 mg/100 g tissue to 987.24 mg/100 g tissue and GSH levels varies from 19.33 nmoles/mg protein to 7.1033 nmoles/mg protein [Table 4].

The three cultivars from each group also showed significant difference in ASH and GSH content. Within each group ASH content varies from 2415.0 mg/100 g tissue to 573.71 mg/100 g tissue and each group GSH content varies from 23.24 nmoles/mg protein to 5.20 nmol/mg protein [Table 5].

3.4. Malondialdehyde (MDA) content

The formation of malondialdehyde (MDA) content was considered as a measure of lipid peroxidation. Duncan's grouping showed that there is no significant difference in MDA content between four groups but showed significant difference within each group where MDA content varies from 11.31 μM/g tissue to 4.44 μM/g tissue [Tables 4 and 5].

3.5. Protein and phenolic content

Duncan grouping showed that there is significant difference in protein and phenolic content between four groups where protein content varies from 13.66 mg/g to 8.28 mg/g, phenolic content varies from 15.36 mg/g to 8.28 mg/g [Table 4].

The three cultivars from each group also showed significant difference in protein and phenolic content. Within each group protein content varies from 16.36 mg/g to 7.10 mg/g, and phenolic content varies from 0.53 mg/g to 0.20 mg/g [Tables 4 and 5].

3.6. Peroxidase isoenzyme pattern

The POD isoforms were analyzed in the 6-days old seedlings of 12 cultivars of pigeon pea by 10% Native-PAGE. The isoenzymes profiles of POD were compared in all 12 cultivars. Seven isoforms of POD were detected, of these six (POD_2-POD_7) were common to all cultivars where as an additional POD band was detected in ICP 15599 [Fig. 1].

4. Discussion

Plants can adapt their growth and developmental processes in response to environmental conditions. Under stress conditions such as drought, high salt, high temperature, and high light intensity, physiological processes are induced to reduce the cellular damage caused by stress, and at the same time, alter developmental

Table 3
Activities of antioxidant enzymes (POD, CAT, SOD and ASO) in seedlings of 12 cultivars with four different durations of flowering time.

Duration	Cultivar name	POD[C] units/mg protein/min (±SD)	CAT[C] units/mg protein/min (±SD)	SOD[C] units/mg protein/min (±SD)	ASO[C] units/mg protein/min (±SD)
Extra-early	Control	0.00 ± 0.00c	0.04 ± 0.00c	2.05 ± 0.08c	2.93 ± 0.10d
	ICP 7220	2.03 + 0.15b	0.43 + 0.01b	4.74 + 0.08b	26.36 + 0.24c
	ICP 15598	2.03 + 0.20b	0.49 + 0.02a	24.37 + 0.50a	28.02 + 0.81b
	ICP 15599	2.73 + 0.20a	0.50 + 0.02a	24.79 + 0.42a	29.44 + 0.62a
		$F = 13.36$[A]	$F = 12.68$[A]	$F = 2643.95$[A]	$F = 19.05$[A]
Early	Control	0.00 ± 0.00d	0.03 ± 0.01c	1.82 ± 0.21d	1.38 ± 0.07c
	ICP 26	0.50 + 0.10c	0.22 + 0.01b	2.90 + 0.09c	12.87 + 0.98b
	ICP 28	1.21 + 0.05b	0.26 + 0.01 ab	13.37 + 0.54b	13.27 + 0.20b
	ICP 1124	1.40 + 0.09a	0.27 + 0.02a	15.29 + 0.32a	16.60 + 0.33a
		$F = 6.28$[A]	$F = 979.40$[A]	$F = 230.56$[A]	$F = 93.00$[A]
Medium	Control	0.00 ± 0.00c	0.04 ± 0.00b	0.82 ± 0.16c	1.19 ± 0.13c
	ICP 472	1.58 + 0.07b	0.35 + 0.02a	3.41 + 0.24b	19.52 + 0.32b
	ICP 3437	1.72 + 0.04a	0.39 + 0.03a	20.82 + 1.00a	19.96 + 0.17 ab
	ICP 7118	1.78 + 0.04a	0.42 + 0.03a	22.02 + 0.64a	20.42 + 0.20a
		$F = 8.65$[A]	$F = 3.17$[B]	$F = 663.42$[A]	$F = 10.20$[A]
Late	Control	0.00 ± 0.00b	0.06 ± 0.00b	1.74 ± 0.25d	1.60 ± 0.33d
	ICP 1342	1.42 + 0.05a	0.34 + 0.03a	3.02 + 0.04c	14.23 + 0.14c
	ICP 1406	1.43 + 0.05a	0.36 + 0.02a	18.48 + 0.37b	15.32 + 0.04b
	ICP 1433	1.44 + 0.01a	0.37 + 0.02a	19.42 + 0.55a	16.92 + 0.32a
		$F = 0.103$[B]	$F = 0.877$[B]	$F = 1692.08$[A]	$F = 128.48$[A]

[A] 5% level of significance ($p < 0.05$).
[B] Not significant.
[C] The values represent the mean ± standard deviation of three independent experiments. Means within a column followed by the same letter are not significantly different from each other at $p = 0.05$ according to Duncan's multiple range test (DMRT).

Table 4
Levels of non-enzymatic antioxidants (ASH, GSH, MDA, phenols and proteins) in seedlings of four different durations of flowering time.

Duration	Levels of non-enzymatic antioxidants				
	ASH[C] mg/100 g (±SD)	GSH[C] nmoles/mg protein (±SD)	MDA[C] content μM/g tissue (±SD)	Phenols[C] mg/g (±SD)	Proteins[C] mg/g (±SD)
Extra-early	1974.11 + 570.98a	19.33 + 5.14a	6.30 + 2.67a	0.38 + 0.11a	8.28 + 1.02d
Early	987.24 + 393.49c	7.10 + 2.15c	8.81 + 1.92a	0.26 + 0.09c	15.36 + 0.85a
Medium	1518.44 + 408.40b	14.34 + 6.61b	7.44 + 2.51a	0.37 + 0.07a	11.22 + 0.83c
Late	1172.78 + 438.92bc	7.17 + 1.20c	8.03 + 2.21a	0.29 + 0.04 ab	13.66 + 2.31b
	$F = 8.06$[A]	$F = 16.76$[A]	$F = 1.82$[B]	$F = 3.61$[A]	$F = 43.53$[A]

[A] 5% level of significance ($p < 0.05$).
[B] Not significant.
[C] The values represent the means (±SD) of three independent experiments. Means within a column followed by the same letter are not significantly different from each other at $p = 0.05$ according to Duncan's multiple range test (DMRT).

timing to complete their life cycle in a timely manner. The effect of stress on flowering time can be ascribed, in part, to induced changes in the epigenome. Flowering in many plant species is regulated by environmental factors, poor nutrition, low temperature and high-intensity light, which can be regarded as stress factors. Several studies suggested that most of the factors responsible for flowering could be regarded as stress.

During the past few years, the complex interrelationship of biochemical pathways that changes during stress has become the important focus. The regulatory roles of the active oxygen species (AOS), detoxification systems in plant abiotic stress tolerance have increasingly attracted much interest because excessive production of AOS is a common consequence of both abiotic and biotic stresses in plants.[26–28]

The levels of enzymatic antioxidants like POD, CAT and ASO showed significant difference between and within the four different flowering cultivars to some extent. But SOD does not showed significant difference. This shows that levels of antioxidant enzymes to some extent are related to flowering time. The absence of significant difference between and within few cultivars might be due same developmental regulation requirements. The difference in the POD activity during early stages of development helps to play an important role in growth and development. Increased CAT activity indicates it removes H_2O_2 produced during β-oxidation of fatty acids during seedling growth as well as flowering. Similarly high ASO activity indicates rapid metabolic activity during seedling growth and flowering which differs from cultivar to cultivar. Similar results were reported in *Lycopersicon esculentum*,[29] *Raphanus sativus*,[30] *Chenopodium rubrum*,[31] *Brassica oleracea*,[32] *Zea mays*.[33,34]

Increased levels of ASH, GSH and low levels of lipid peroxidation indicate the inhibitory effect of ASH/GSH on lipid peroxidation. Similar results were reported in tomato by Shalata and Neumann,[35] and also in radish.[30] The results of the present study showed that ROS levels are controlled via a versatile antioxidant network in plants. The specific interplay between ROS and components of the antioxidant pathways could generate compartment-specific changes in both the absolute concentrations of ROS, antioxidant compounds as well as in ascorbate and glutathione redox ratios. Under stress conditions, these redox signals could interfere with the signaling networks complementary to the antioxidant system and regulate defense gene expression, thus coordinating the necessary readjustments in the redox-regulated plant defense to overcome oxidative stress.

Total phenolic content as well as protein content differs between and within groups. Increased phenolic content corresponds to increased antioxidant activity. Similar results on phenolics mobilization during seed germination of *Pangium edule* Reinw was reported by Andarwulan et al. [36]

Table 5
Levels of non-enzymatic antioxidants (ASH, GSH, MDA, phenols and proteins) in seedlings of 12 cultivars with four different durations of flowering time.

Duration	Cultivar name	ASH[C] mg/100 g (±SD)	GSH[C] nmoles/mg protein (±SD)	MDA[C] content μM/g tissue (±SD)	Phenols[C] mg/g (±SD)	Proteins[C] mg/g (±SD)
Extra-early	Control	185.33 ± 11.68d	2.05 ± 0.22d	0.55 ± 0.1c	0.06 ± 0.02c	6.58 ± 0.18d
	ICP 7220	1216.67 + 10.40c	12.50 + 0.14c	9.86 + 0.10a	0.30 + 0.02b	7.10 + 0.20c
	ICP 15598	2415.00 + 15.87a	23.24 + 0.08a	4.60 + 0.16b	0.30 + 0.01b	9.40 + 0.40a
	ICP 15599	2290.67 + 35.38b	22.26 + 0.31b	4.44 + 0.06b	0.53 + 0.07a	8.34 + 0.26b
		$F = 2422.94$[A]	$F = 2522.64$[A]	$F = 2022.42$[A]	$F = 22.11$[A]	$F = 44.02$[A]
Early	Control	111.33 ± 14.82d	2.23 ± 0.15d	1.41 ± 0.16d	0.02 ± 0.01c	10.60 ± 0.18d
	ICP 26	573.71 + 7.20c	5.20 + 0.17c	11.31 + 0.38a	0.20 + 0.01b	16.36 + 0.46a
	ICP 28	914.67 + 13.65b	6.19 + 0.15b	8.00 + 0.40b	0.20 + 0.03b	15.25 + 0.06b
	ICP 1124	1473.33 + 16.65a	9.90 + 0.09a	7.11 + 0.12c	0.39 + 0.02a	14.48 + 0.20c
		$F = 3600.97$[A]	$F = 891.11$[A]	$F = 272.27$[A]	$F = 60.17$[A]	$F = 30.74$[A]
Medium	Control	136.33 ± 10.26d	0.11 ± 0.03d	0.67 ± 0.03d	0.02 ± 0.01c	6.64 ± 0.36c
	ICP 472	975.67 + 10.50c	6.26 + 0.10c	10.77 + 0.21a	0.30 + 0.01b	11.85 + 0.10a
	ICP 3437	1756.67 + 26.57b	15.32 + 0.18b	6.02 + 0.04b	0.34 + 0.03b	11.65 + 0.36a
	ICP 7118	1823.00 + 14.10a	21.43 + 0.37a	5.54 + 0.33c	0.46 + 0.02a	10.15 + 0.15b
		$F = 1967.67$[A]	$F = 2845.81$[A]	$F = 483.56$[A]	$F = 34.35$[A]	$F = 46.60$[A]
Late	Control	165.00 ± 26.46c	0.65 ± 0.01c	0.72 ± 0.09d	0.05 ± 0.03c	8.30 ± 0.33d
	ICP 1342	588.00 + 2.00b	6.21 + 0.05b	10.94 + 0.02a	0.26 + 0.01b	15.50 + 0.25a
	ICP 1406	1453.00 + 24.51a	6.54 + 0.10b	7.04 + 0.15b	0.26 + 0.03b	14.88 + 0.72b
	ICP 1433	1477.33 + 12.85a	8.75 + 0.33a	6.12 + 0.11c	0.35 + 0.02a	10.60 + 0.09c
		$F = 2998.17$[A]	$F = 137.80$[A]	$F = 1654.54$[A]	$F = 14.64$[A]	$F = 831.71$[A]

[A] 5% level of significance ($p < 0.05$).
[C] The values represent the mean ± standard deviation of three independent experiments. Means within a column followed by the same letter are not significantly different from each other at $p = 0.05$ according to Duncan's multiple range test (DMRT).

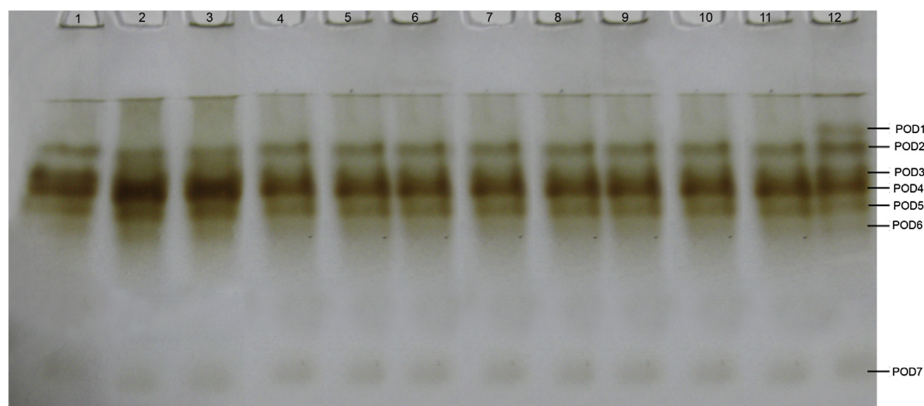

Fig. 1. Peroxidase isoenzyme pattern in 6 days old seedlings of 12 cultivars of pigeon pea.
1, 5, 9: ICP 26, ICP 28, and ICP 1124 (early duration)
2, 6, 10: ICP 472, ICP 3437, and ICP 7118 (medium duration)
3, 7, 11: ICP 1342, ICP 1403, and ICP 1433 (late duration)
4, 8, 12: ICP 7220, ICP 15598, and ICP 15599 (extra-early duration)

4.1. Peroxidase isozyme pattern

All 12 cultivars showed six common isoforms (POD_2 to POD_7), but an additional isoform (POD_1) in extra-early variety might be a specific marker for that variety. This shows the role of peroxidase and its isoforms in the early stages of seedling growth in pigeon pea. These results are in agreement with the findings of Jackson and Ricardo[37] in the early stages of lupin growth, in tomato seeds by Morohashi[29] and during germination of *Viola cornuta* seeds.[38] The role of multiple isoenzymes of POD was also explained by Duroux and Welinder.[39]

5. Conclusion

Among four different flowering durations extra-early group showed highest activity of enzymatic and non-enzymatic antioxidants, whereas lipid peroxidation was least. This shows lipid peroxidation has adverse effects on flowering of plants. In extra-early duration varieties due to least lipid peroxidation flowering might be faster when compare to other varieties. Similarly increased levels of antioxidants also played a major role in avoiding stress to the plant hence flowering at early stages was observed. The isozyme analysis of peroxidase in the present study shows there is no specific isoform for flowering time identification, but however there is one marker for extra-early cultivar (ICP 15599).

Conflicts of interest

All authors have none to declare.

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