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Influence of Accelerated Ageing on Total Soluble Seed Protein Profiles of Tomato

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ABSTRACT Study was conducted to compare 0 to 9 days accelerated aged seed lots of tomato with germination and vigour index that varied from 92 to 0 and 1210 to 0, respectively. Germination loss became more accentuated with increase in time of ageing. Total soluble seed protein banding pattern of different aged seeds revealed that there has been decline in band intensity, band numbers or disappearance of some bands as period of ageing advanced. However, no much variation was observed up to three days of ageing (i.e. up to 41 % of germination). Thus, seed lots with slight variation either in germination or vigour could also be used for varietal characterization by SDS-PAGE to differentiate the cultivars or even for genetic purity testing, but not the seed lots which are severely aged that lost threshold limit of 50 per cent.

Key words: Tomato seed, accelerated ageing, SDS-PAGE, protein profiles

Recent findings in biochemistry and molecular biology have enabled seed scientists to utilize new techniques for cultivar identification to augment existing traditional methods (Grow-Out-Test). Proteins are direct products of structural genes and are independent of environmental factors; these markers have been used to characterize varieties and to test the hybrid purity of many important horticultural crops [1, 2, 3 & 4]. Seed is the best material to extract protein because examination can be carried out immediately after or even before harvest.

It is very essential to know the effect of ageing on seed protein profiles so that an appropriate age of seed can be considered for electrophoretic analysis of seed proteins to characterize and differentiate the cultivars. Hence, an attempt was made to find out threshold limit for seed ageing, up to which these can be used for electrophoretic analysis of proteins.

MATERIALS AND METHODS

Seeds of tomato cv. Pusa Ruby were subjected to accelerated ageing at 40±1°C and 90 per cent RH for a period of 1 to 9 days in order to obtain the seed lots of varying quality in terms of viability and vigour (V_1, V_2, V_3, V_4, V_5, V_6, V_7, V_8 and V_9). Seeds were then bench dried to the original moisture content. Seeds not exposed to ageing conditions were considered as control (V_0). Aged as well as non aged seed lots were evaluated for germination as per ISTA [5] and seed vigour index [6]. Further, SDS-PAGE of total soluble proteins of all the seed lots was carried out by using 12 per cent acrylamide gel according to the methods prescribed by Laemeli [7] with slight modifications. Five seeds were ground in 200ml Tris-HCl extraction buffer (25 mM, pH 8.8) in centrifuge tube. The mixture was mixed thoroughly and kept over night at 8°C for protein extraction. Then the mixture was centrifuged at 10,000 rpm for 15 minutes and the supernatant was collected. This

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protein extract was dissolved in an equal volume of working buffer (0.06 M Tris-HCl, pH 6.8, 2% SDS, 10 % glycerol, 0.025 % bromophenol blue) and incubated at 60-70°C for 10 minutes on dry bath, cooled immediately for 5 minutes and centrifuged at 10,000 rpm for 5 minutes. The supernatant was used for loading in the gel. A current of 1.5 mA per well with a voltage of 80 V was applied until the tracking dye crossed the stacking gel. Later the current was increased to 2 mA per well and voltage up to 120 V. The electrophoresis was stopped when the tracking dye reached the bottom of the resolving gel. Then the gel was stained using 1 per cent coomaasie brilliant blue solution overnight and destained using a mixture of 227 ml of methanol, 46 ml of acetic acid and 227 ml of distilled water until the bands were clearly visible.

RESULTS AND DISCUSSION

Seeds attain maximum quality at physiological maturity. Starting from this point, there is a series of degenerative events that reduce the survival capacity of seeds and lead to loss of vigour and germination [8]. Main sites of ageing at cellular level are mitochondria, ribosomes and membranes. Ageing process is mainly due to reduction in enzymatic activity; increased respiration and macromolecule synthesis, which are associated with initial deterioration of membrane system. A considerable amount of work has been done on the changes in protein content [9, 10, 11 & 12] and changes in activity of proteolytic enzymes [13, 14, 15 & 16] related to seed deterioration. However, there is a need to know whether the protein profiles, the qualitative aspects of protein is going to alter due to ageing that would help to select the right age of seed lots for varietal characterization since the protein profiles are being used to identify the off types at the seed level.

In the present study, accelerated ageing affects germination and vigour of the seeds. Germination loss become more accentuated with progressive ageing (Table 1), it varied from 92 to zero per cent from non aged to aged lots (9 days), respectively. Accordingly, vigour index also varied and was highest in control (1210) and decreased with ageing.

Table 1. Seed vigour levels created by accelerated ageing

Seed lots	Period of ageing (days)	Germination	Vigour Index
V_0	0	92	1210
V_1	1	83	900
V_2	2	72	740
V_3	3	41	367
V_4	4	33	166
V_5	5	16	76
V_6	6	3	-
V_7	7	1	-
V_8	8	0.1	-
V_9	9	0.0	-

When the total soluble seed protein banding pattern of different aged seed lots was compared, in general there was decline in band intensity, band numbers or disappearance of some bands as period of ageing advanced, i.e., highest molecular weight subunits were disintegrated into low molecular weight subunits. This was mainly due to degradation of protein in aged seed lots resulting in reduction of band intensity or disappearance of protein bands. Several researchers also reported such degradation of proteins in terms of reduction in number and intensity of bands with increased period of seed age [17, 18 & 11].

In our study, there was decreased band intensity or total disappearance of a particular band as ageing period progressed. However, no such variation was observed in first four aged seed lots (V_0, V_1, V_2 and V_3) except for 14^{th} band (Rm: 0.589), which represents 21 KD proteins. Even though, three days aged seed lot showed 41 per cent germination, which was below minimum seed certification standard, had similar protein profiles as that of non-aged seeds except for 14^{th} band (Rm: 0.589) (Table 2, Figs. 1 & 2). This was the only protein band degraded after few days of ageing and that can be considered as susceptible protein for ageing process. Krishnasamy and Yugasandhya [16] also did not notice any difference in protein profiles of aged and non-aged seeds of maize. However, in this study, it was not

Table 2. Intensity and relative mobility of total soluble seed proteins of different aged seed lots of tomato cv. Pusa Ruby

Band No.	Rm value	V_0	V_1	V_2	V_3	V_4	V_5	V_6	V_7	V_8	V_9
1	0.101	+	+	+	+	+	+	+	+	+	+
2	0.112	++	++	++	++	++	++	++	++	+	-
3	0.134	+	+	+	+	-	-	-	-	-	-
4	0.146	+	+	+	+	+	+	+	-	-	-
5	0.157	++	++	++	++	++	++	++	+	+	+
6	0.186	++	++	++	++	++	++	++	+	+	+
7	0.213	++	++	++	++	++	++	++	+		
8	0.241	++	++	++	++	++	++	++	+	+	+
9	0.275	+	+	+	+	+	+	+	+	-	-
10	0.325	+	+	+	+	+	+	-	-	-	-
11	0.359	+++	+++	+++	+++	+++	+++	+++	+++	+	+
12	0.382	++	++	++	++	++	++	++	+	+	+
13	0.550	++	+	+	+	+	+	+	+	-	-
14	0.589	++	-	-	-	-	-	-	-	-	-
15	0.653	+	+	+	+	+	+	+	+	+	+

clear how this particular seed protein was degraded. Hence, there is need to study the mechanism of degradation of this protein. Thus, seed lots with slight variation either in germination or vigour could also be used for varietal characterization by SDS-PAGE to differentiate the cultivars or even for genetic purity testing but not the seed lots which are more aged that lost threshold limit of 50 per cent.

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