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Influence of packaging material and storage time on seed germination and chromosome biology of inbred line of maize (*Zea mays* L.)

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This present study was elucidated the cytological effects of maize due to ageing (storage) in different packaging material. This study revealed that the cytological anomalies in somatic cells of maize inbreds (CM-138 and CM-142) due to storage in jute and plastic bags. For the cytological impact of storage, inbreds were stored in jute and plastic bags for different periods i.e. 4, 8 and 12 months under ambient and control conditions of Allahabad. Observations showed that inbreds stored in plastic bags of eight and twelve months ageing treatments displayed more mitotic anomalies in comparison to four month aged seeds of jute bags. The major chromosomal anomalies included stickiness of chromosomes, fragments, laggards, disturbed prophase etc. The frequencies of anomalies were higher in the twelve months aged seeds of plastic bags as compared to jute bags. In both the packaging materials, seedling parameters were also found more affected in plastic bags as compared to jute bags in both the inbred lines of maize. Germination and vigour percentage decreased with the period of ageing. Inbred are stored in plastic bags were affected due to storage but the effects were more pronounced in the plastic bags as compared to jute bags.

Key Words: Packaging material, Storage, Maize inbred, chromosomal abnormality.

Introduction

Maize is one of the most important cereal crops in the world agricultural economy both as food for man and feed for animal. In Indian Agriculture, Maize occupies a prominent position and each part of the maize plant is put to one or the other use and nothing goes as waste. Maize as a crop has multiple uses but is chiefly grown for human and Livestock consumption. The seeds and

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the cobs are used as basic raw material in various industries. The seeds are processed and converted into needed preparations, flakes, grits and pops for human consumption (Kumar and Rai, 2006).

Seeds are required to be kept in safe storage since they are harvested in the preceding season and usually used for sowing in the subsequent season often after a time gap of six months or longer. Thus, proper storage is required to keep seeds in good condition. Some varieties needs air conditioned storage. Storage costs are also added in order to drive cost of seeds.

Seed is stored from the moment it attains physiological maturity on the plant until it is sown. The weathering agencies like high moisture; oxygen, sunlight, insects and diseases cause adverse effects on the seeds before harvest. Improper and delayed harvesting as well as processing cause further injury to the seeds. Seeds are stored under optimal storage conditions (low temperature and low seed moisture content) to prolong the seed viability. The deterioration of stored seed is a natural phenomenon and the seeds tend to loose viability even under ideal storage conditions. Ageing is an universal physiological phenomenon occurring in living organisms. It is one of the most intriguing and challenging scientific problems of universal concern. The poor seed storability is a major problem in maize. The changes associated with seed deterioration are manifested in various seed and seedling characters at different stages. Among these deteriorative changes, membrane degradation has been proposed as the primary event in ageing (Dadlani and Agarwal, 1983; Varghese and Rai, 2005). Hence, present studies were undertaken to assess the cytological changes with deterioration of maize seeds and the effect of storage bags on prolonging/maintaining its longevity under ambient and control conditions of Allahabad.

Materials and methods

Seeds of inbred line of maize, i.e. CM-138 and CM-142 were obtained from Division of Genetics, Indian Agricultural Research Institute (I.A.R.I.), New Delhi. Inbred were stored in jute and plastic bags for different periods, i.e. 4, 8 and 12 months under ambient and control conditions of Allahabad (Plants Genetics Laboratory). The treated seeds and control seeds (fresh seeds) were grown in petridishes in Plants Genetics Laboratory same day after storage treatments with three replications. The petridishes were placed in incubator maintained at 25 ± 1^0C. For each treatment ten seeds were used in each replication. The seeds from each sample were then germinated on moist filter paper in petridishes. The excised root tips were first given pre-treatment in 0.1% colchicines solution for about 4 hours at room temperature for maximizing the division of cells. After that, root tips were washed in distilled

water and fixed in a fresh solution (3:1) of ethyl alcohol and acetic acid. Slides were prepared using chromosome squash technique with 2% acetocarmine.

For seedling characters, the germination test was conducted using three replications of 100 seeds from each sample in rolled towel papers as per procedure described by ISTA (1993). Seedling dry weight and vigour index I and II were determined by Baki and Anderson (1973).

Data analysis

In order to calculate the Germination (%), Vigour Index (I and II), Root and Shoot length, Seedling Dry Weight and Mitotic Index (M.I.) formula 1, formula 2, formula 3, formula 4 and formula 5 were used:

$$\text{Germination (\%)} = \frac{\text{Number of normal seedling}}{\text{Total seeds used for germination test}} \times 100 \quad (1)$$

V.I. (I) = Germination percentage (Normal seedling) X Seedling length (cm) (2)

V. I. (II) = Germination percentage (Normal seedling) X Dry weight of the seedling (gm)

Root and shoot length: Root and shoot length of five fresh seedlings was measured in centimeters up to one decimal. Total seedling length was calculated by adding root and shoot length. (3)

Seedling dry weight: The seedlings used for recording were dried in an oven at $103^0C \pm 1^0C$ for 12 hours. Measurement of dried samples was record on an electronic balance upto three decimals in mg. (4)

$$\text{M. I. (\%)} = \frac{\text{Number of dividing cells}}{\text{Total number of cells observed}} \times 100 \quad (5)$$

Results

Seeds of inbred line of maize, i.e. CM-138 and CM-142 were cytologically examined for mitotic abnormalities due to packaging materials. Data were recorded from well spread cells under 40x microscopic magnification. The somatic chromosome number was found to be 2n=20. In the control sets, mitosis was found to be normal with a mitotic index of 15.71%±0.58 in CM-138 and 14.95%±1.17 in CM-142, while in the seeds stored in jute and plastic bags. However, in the ageing treatment sets, a clearcut mito-depressive and chromotoxic effect was recorded in both the packaging materials but the effect was much more pronounced in CM-142 as compared to CM-138. Maximum reduction in mitotic index was recorded in twelve month of

stored seeds in plastic bags in both the inbred line, i.e. 12.69% ±0.49 in CM-138 and 12.18%±1.45 in CM-142 as compared to jute bags, i.e. 13.05%±0.68 in CM-138 and 12.67%±0.91 in CM-142, respectively.

A number of chromosomal anomalies have also been recorded in the treated sets, viz. stickiness, scattering, precocious movements, fragments, laggard and bridges (Table 1). It was observed that the abnormality percentage increased along with the increase in duration of ageing treatments in both the packaging materials.

A maximum chromosomal abnormality was encountered in seeds stored in plastic bags for 12 months in both the inbred line, i.e. 7.03%±0.26 in CM-138 and 8.56%±0.29 in CM-142. Most frequent abnormality observed among the ageing treatment sets was stickiness was recorded to be as high as 1±0.10 at jute bags and 2±1.02 at plastic bags in the seeds stored for 12 months while bridges and laggard were some of the least frequent abnormalities. Both the storage devices were affected the inbred line due to ageing but the effects were more pronounced in the seeds stored in plastic bags compared to jute bags.

Ageing treatments also affected the seedling characters of the treated sets. Germination percentage was found to be maximum (93.5%±0.33) in the control set of CM-138, while the minimum germination percentage (92.5%±0.79) was observed in the inbred line CM-142. It was observed that, along with increasing treatment duration of ageing, the germination percentage was reduced continuously. Similar trends in root length, shoot length, seedling length, seedling dry weight, vigour index I and II were recorded after ageing treatments in both packaging materials (Table 2). In seedling parameters, seed stored in jute bags gives better performance in the comparison of seeds stored in plastic bags.

Discussion

In modern agriculture, seed deterioration under storage is a problem (Nutile, 1964). Ageing process is affected by the genetic factors (Crocker and Barton, 1957; Varghese and Rai, 2005). Mitotic index values could be reduced due to ageing in the root meristems of maize; which can also be attributed to mitotic inhibitions. Mitotic inhibition by ageing can be attributed to blocking of mitotic cycle, which may result from prolonged G_2 period or to defective DNA synthesis. Cytological observations of dividing cells revealed an abundance of chromosomal irregularities, which were directly proportional to the durations of ageing treatment. The prominent irregularities observed in both the inbred line after storage in jute and plastic bags were stickiness, precocious movements of the chromosomes, scattering, binuclete cells, laggards and bridges etc.

The chromosomal aberrations in the root meristems of the seeds also indicate that the ageing somehow alters the normal structure and function of chromosome. Earlier researchers have reported the increased chromosomal aberrations along with the increase in the storage periods of seeds from a wide range of species e.g. *Pisum sativum* L. (D' Amato, 1951) and in *Zea mays* L. (Berjak, 1968; Varghese and Rai, 2005; Kumar and Rai, 2006; Kumar and Rai 2007; Kumar and Rai, 2009). It has made a fresh approach in defining the pattern of seed ageing in establishing the relationship between rate of ageing and chromosome damage. Although, chromosomal stickiness is one of the phenomena in chromosomal behaviour that has been recorded for almost a century, adequate explanations are still lacking. The phenomenon was earlier identified by Koernicke (1905) and is characterized by intense chromosome clustering during any phase of cell cycle. The term stickiness was firstly employed by Beadle (1932) when he described the sticky aspect of chromosomes in maize cell that had suffered a mutation. Chromosome stickiness has been documented to be due to genetic or environmental factors. Genetically induced stickiness was reported in *Zea mays* L. (Caetano-Pereira *et al.,* 1995; Varghese and Rai, 2005; Kumar and Rai, 2006; Kumar and Rai, 2007; Kumar and Rai, 2009) while stickiness has also been reported in other crops like wheat (Zanella *et al.,* 1991) and in millet (Rao *et al.,* 1990), *Glycine max* (Bione e*t al.,* 2000). Gaulden (1987) postulated that sticky chromosomes might result from the defective functioning of one or two types of specific non-histone proteins involved in chromosome organization, which are needed for chromatid separation and segregation. The formation of bridges could be attributed to chromosomal stickiness (El-Khodary, 1989) and to chromosome breakage and reunion (Haliem *et al.,* 1990; Kumar and Rai, 2006). Induction of bridges and breaks may lead to the loss of genetic material (Salem *et al.,* 1993, Kumar and Rai, 2006). Binuclete cells might have formed due to the inhibition of cell wall development at telophase. The movement of the chromosomes ahead of the rest might have cause precocious movement during anaphase (Permjit and Grover, 1985). The reduction in mitotic index indicates that the ageing might have an adverse effect on the mitotic apparatus.

Several workers reported that during ageing of seed under storage, chromosomal aberrations that consisted of mostly bridges, laggards, fragments, disturbed anaphase, stickiness, micronuclei like chromatin fragments and point mutations occur in various crops (Kumar, 1988; Purkar, 1980; Purkar, 1980b; Varghese and Rai, 2005; Kumar and Rai, 2006; Kumar and Rai, 2007; Kumar and Rai, 2009).

Chromosomal damages may be the prominent causes of reduced germination and other seedling characters as compared to control. The

reduction in germination percentage might have been due to the effect of storage on meristematic tissues of the seed. The ageing treatments also delayed the germination process. Kelinhofs *et al.* (1978) reported a delay in the initiation of metabolism following germination, resulting in uniform delay in mitotic activity, seedling growth and ATP and DNA synthesis.

Krishnaveni (1984) and German *et al.* (1993) while working on maize reported a significant reduction in germination, viability, root length, dry matter reduction and vigour index with response to period of ageing. Similar results were also obtained by Abdulla and Roberts (1969), Dharmalingam *et al.* (1976); Ravichandran (1991); Singh *et al.* (2003), Varghese and Rai (2005); Kumar and Rai (2006); Kumar and Rai (2007); Kumar and Rai (2009).

As a result of these studies, genetic segregations should be carefully observed. From the present study, it is suggest that the seed stored in jute bags enhances the storage life of maize seeds as compared to plastic bags. Seeds of inbred CM-138 showed better storability as compared with inbred CM-142.

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Table 1. Effect of different storage containers on chromosomal anomalies of inbred line of *Zea mays* L. CM-138 and CM-142

I. L.	Packaging material	Ageing treatment (In months)	Number of Observed cells	Number of dividing cells	M. I. (%) Mean± S.E.	St. Mean± S.E.	Sc. Mean± S.E.	P.M. Mean± S.E.	Mic. Mean± S.E.	Fr. Mean± S.E.	Lg. Mean± S.E.	Br. Mean± S.E.	*Oth. Ab. Mean± S.E.	T Ab (%) Mean± S.E.
CM-138	Jute Bags	Control	1642	258	15.71±0.58	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		A_4	1628	240	14.74±0.67	0.00	1±0.10	0.00	0.00	0.00	0.00	0.00	0.00	1.76± 0.12
		A_8	1652	234	14.16±0.54	1±0.11	1±0.17	0.00	1±0.16	1±0.13	1±0.12	0.00	1±0.13	3.45± 0.17
		A_{12}	1647	215	13.05±0.68	2±0.16	0.00	1±0.17	0.00	1±0.19	2±0.19	1±0.11	2±0.15	5.78± 0.21
	Plastic Bags	A_4	1624	226	13.91±0.72	1±0.18	1±0.06	0.00	0.00	0.00	0.00	0.00	0.00	2.03± 0.16
		A_8	1578	213	13.49±0.57	1±0.24	1±0.14	0.00	1±0.17	0.00	1±0.11	0.00	2±0.18	4.98± 0.21
		A_{12}	1630	207	12.69±0.49	2±0.29	0.00	2±0.28	2±0.30	1±0.24	1±0.17	1±0.19	2±0.21	7.03± 0.26
CM-142	Jute Bags	Control	1658	248	14.95±1.17	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
		A_4	1578	220	13.96±0.69	1±0.07	1±0.19	1±0.14	0.00	0.00	0.00	0.00	0.00	2.08± 0.15
		A_8	1557	203	13.03±0.78	1±0.16	2±0.27	0.00	1±0.21	1±0.17	0.00	0.00	1±0.15	5.98± 0.19
		A_{12}	1562	198	12.67±0.91	2±0.34	1±0.29	2±0.27	1±0.26	2±0.15	0.00	1±0.21	2±0.23	7.02± 0.23
	Plastic Bags	A_4	1597	213	13.33±0.86	1±0.13	1±0.16	0.00	0.00	0.00	0.00	0.00	1±0.16	3.08± 0.17
		A_8	1582	203	12.83±1.26	1±0.24	1±0.21	1±0.16	0.00	1±0.19	1±0.21	0.00	2±0.24	6.89± 0.25
		A_{12}	1567	191	12.18±1.45	2±0.39	2±0.29	2±0.25	2±0.29	2±0.28	2±0.25	2±0.27	2±0.31	8.56± 0.29

Legends of Table 1

I. L.- Inbred Lines, M.I.- Mitotic Index, Tab- Total abnormalities, A_4- Four month aged seeds, A_8- Eight month aged seeds, A_{12}- Twelve month aged seeds, St.- Stickiness, Sc- Scattering, P.M.- Precocious movement, L - Laggards, B - Bridges, U - Unorientation, Others-*Fragmentation, Forward movement, Micronuclei, Nonsynchronous division, S. E. - Standard error.

Table 2. Effect of different storage containers on seedling parameters of inbred line of *Zea mays* L. CM-138 and CM-142

I. L.	Packaging material	Ageing Treatments (In months)	Germination (%) Mean±S.E.	V. I. (I)	V. I.(II) Mean±S.E.	Root length (In cm) Mean±S.E.	Shoot length (In cm) Mean±S.E.	Seedling Length (In cm) Mean±S.E.	Seedling Dry Weight (gm) Mean
CM-138	Jute Bags	Control	93.5±0.33	3324	33.11±0.98	19.95±0.45	20.73±1.07	38.67±1.07	0.46
		A_4	91.6±1.08	3143	29.71±0.78	17.37±0.56	18.76±0.93	33.23±0.89	0.41
		A_8	85.1±0.97	2934	27.14±0.65	15.21±0.67	16.89±0.85	30.12±0.68	0.39
		A_{12}	80.2±0.67	2613	23.59±0.78	13.39±0.49	13.98±0.56	27.32±0.82	0.37
	Plastic Bags	A_4	89.1±0.35	2995	27.45±0.82	15.65±0.56	15.64±0.39	30.29±0.69	0.39
		A_8	81.6±0.56	2851	23.12±0.45	13.78±0.78	11.89±0.73	28.32±0.71	0.36
		A_{12}	77.3±0.27	2513	19.11±0.34	11.43±1.08	10.01±0.34	25.32±0.53	0.33
CM-142	Jute Bags	Control	92.5±0.79	3299	30.23±1.01	18.79±1.06	19.96±0.42	35.56±0.49	0.43
		A_4	90.6±1.02	3129	27.87±0.76	16.15±0.97	17.78±0.59	31.29±0.55	0.40
		A_8	83.1±0.97	2906	24.89±0.56	14.22±0.59	14.34±0.93	27.89±0.68	0.36
		A_{12}	80.8±0.86	2597	21.67±0.89	11.79±0.79	12.76±0.71	25.78±0.35	0.31
	Plastic Bags	A_4	87.1±0.49	2957	25.45±0.76	14.78±0.73	14.34±0.57	29.45±0.71	0.37
		A_8	79.5±0.39	2799	21.67±0.56	10.98±0.81	11.98±0.68	26.56±0.58	0.34
		A_{12}	75.4±0.73	2498	18.23±0.45	9.01±0.39	9.45±0.51	24.67±0.35	0.31

Legends of Table 2

I. L. - Inbred Lines, A_4- Four month aged seeds, A_8- Eight month aged seeds, A_{12}- Twelve month aged seeds, V. I. – Vigour Index, S. E. - Standard error.