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# Seed development and acquisition of desiccation tolerance during maturation of okra seed

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**ABSTRACT**

**Studies on seed development and maturation were conducted during *kharif* 2008. Okra seeds of Pusa A-4 cultivar were harvested 10, 17, 24, 28, 32, 36 and 40 days after anthesis (DAA). Seeds were immediately shelled by hand and a part of freshly harvested seeds were analyzed for moisture content, leachate conductivity, seed dry weight and seed germination. Other part of the freshly harvested seeds at each sampling date was subjected to rapid desiccation to identify the stage at which desiccation tolerance occurred. With the advancement of seed development, seed coat colour changed from white to grey. Maximum hard seeds were found when seed coat colour changed to grey at 40 days after anthesis. No seeds germinated until 17 DAA. As soon as seeds began to germinate at 28 DAA, the percentage of hard seeds increased as seed moisture content decreased. There were no hard seed in fresh seeds and rapidly dried seeds with the higher moisture content till 24 DAA. With the advancement of seed development there was a drastic reduction in moisture content and the occurrence of hard seeds were observed. Maximum hard seeds were found when seed moisture content was minimum at 40 days after anthesis in fresh developing and rapidly dried seeds. Maximum seed germination in fresh developing seeds was attained at 36 DAA, when physiological maturity and maximum seed dry weight were attained. With seed maturation, there were rapid decline in leachate conductivity and seed moisture content. Total soluble sugars increased from 10 to 36 DAA and non-reducing sugars increased drastically at 28 DAA, which was concomitant to the stage at which desiccation tolerance occurred.**

**Key words:** Desiccation tolerance, okra seed development, maturation.

## INTRODUCTION

Seeds proceed through embryogenesis, maturation and desiccation after fertilization and at each stage different physiological and biochemical changes occur to accommodate the requirements of germination (Bewley and Black, 3). Seed development is also affected by many factors including genetic, physiological and environmental elements. In many orthodox seeds, maximum dry weight occurs soon after the mid-point of development and is preceded by a cessation of net increase in the amount of water per seed. Consequently, continued deposition of storage materials within seed results in decrease in seed moisture from around 80% to about 40% on fresh weight basis at the time of maximum seed weight basis (Welbaum and Bradford, 25). Mature orthodox seeds are defined as having the ability to tolerate extreme desiccation. This serves as an adaptive mechanism for seeds to survive severe environmental stresses and to preserve their viability during long storage (Leprince *et al.*, 16). In indeterminate crops like okra, early harvest results in immature seed of low vigour, whereas late harvest risk seed deterioration and seed loss due to shattering. In grasses and small seeded legumes, Klein and Harmond (15) showed that the optimum time for realising maximum yields precedes the period of highest germination. In some cases highest germination had occurred after some shattering had taken place. Accordingly, the optimum time of harvest is a result of the balance between seed weight, viability and shatter loss. The presence of hard seed coat in okra is a major physiological constraint to rapid and uniform stand establishment in the field. Some of the most recent studies reported an interrelation between seed moisture content and hard seededness. Several reports indicate that the percentage of hard seeds showed a progressive decline with increasing moisture content. Ellis *et al.* (10) reported little or no hard seededness at 13% but a considerable amount at 4-6% moisture content. Seed producers often leave the seeds in pods until they dry completely to below 10% moisture level resulting in higher proportion of hard seeds in the seed lot. The objective of the present research was to find out interrelation of seed moisture with occurrence of hard seededness in okra and relate changes in physiological and biochemical parameters with the timing of desiccation tolerance during seed development.

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## MATERIALS AND METHODS

A field experiment was conducted at IARI Experimental Farm, New Delhi, during summer 2008. The okra crop of Pusa A-4 variety was sown on 2nd July, 2008 in an area 500 $m^2$ for seed production. The sowing was done on ridges at a spacing of 60 cm × 30 cm following recommended cultural practices and plant protection measures. Flowers were tagged on the day of anthesis at 3rd node from the base on individual plants and approximately 500 flowers were tagged. Fruits were harvested 10, 17, 24, 28, 32, 36 and 40 days after anthesis (till maturity). On each sampling date 20 pods were harvested and seeds were shelled by hand. The bulked seed was divided into two parts. Half quantity of the freshly harvested seeds was used for analysis and other half quantity of the pooled seed was rapidly dried. Rapid drying was performed by equilibrating fresh seed over saturated solution of $LiCl_2$ at 25°C giving 13% RH for 6 days to determine their desiccation tolerance. Seeds were humidified over water for 24 h and then tested for germination.

Seed coat colour of okra was determined by using RHS colour chart. To determine the seed water content, three replicates of 50 seeds each were weighed in a weighing bottle for obtaining fresh weight and dry weight was obtained after drying in an oven at 130°C for 1 h (Anon., 2). The germination test was conducted by between paper (BP) method where four replicates of 100-seed each were tested for following ISTA method (Anon., 2). Three replicates of 25-seed were soaked in 40 ml of deionized water for 24 h. Seed leachate was collected and conductivity was measured using an electrical conductivity bridge as $\mu S\ cm^{-1}g^{-1}$ of seed. EC of distilled water was taken as control.

The content of the reducing sugars and total soluble carbohydrates in seeds was determined using pre-weighed and frozen samples, which were ground in a mortar and pestle with 5 ml 80% ethanol and made up to a volume of 10 ml after refluxing in hot water for two hours. The sample was centrifuged for 15 min. at 4000 rpm and the supernatant was used. To 5 ml of the supernatant, 0.5 ml concentrated HCl was added and incubated overnight at room temperature to hydrolyze the sugars. The volume was then adjusted to 25 ml and aliquots were taken to estimate total soluble sugars using anthrone reagent and glucose as standard (Hodge and Hofreiter, 13). The other half of the supernatant was directly used for estimation of reducing sugars by the same method. The difference gave the non-reducing sugar content in the samples.

## RESULTS AND DISCUSSION

Seeds harvested at 10 days after anthesis showed white group seed coat colour (Table 1). With the advancement of seed development the seed coat color changed to yellow-white group (5%). After 28 days of anthesis, 78% seeds showed seed coat colour of yellow-white group and 22% showed grey-yellow colour. After 32 days of anthesis 100% seeds showed seed coat colour of grey group, which persisted up to last date of harvesting (40 DAA). Demir (8) also reported that increases in number of black seeds in okra cv. Akkoy were associated with increase in hard seeds. However, the highest number of hard seeds were recorded when 100% of seeds were black.

No hard seed were found with white seed coat colour but as seed coat colour started turning to grey-yellow, the occurrence of hard seeds were observed (8.75%). Maximum hard seeds were found when seed coat colour changed to Grey group starting 1.75% at 32 days after anthesis to 20% at 40 days after anthesis in fresh developing seeds. Hard seededness is a major

**Table 1.** Seed coat colour of freshly harvested seeds during seed development and its relationship with occurance of hard seededness in okra.

| Days after anthesis | Seed coat colour | Percentage | Hard seed (%) |
|---|---|---|---|
| 10 | White group 155 | 100 | 0 |
| 17 | White group 155A | 100 | 0 |
| 24 | White group 155D | 95 | 0 |
| | Yellow-white group 158B | 5 | |
| 28 | Yellow-white group 158B | 78 | 8.75 |
| | Grey-yellow group 161B | 22 | |
| 32 | Grey group 200C | 100 | 11.75 |
| 36 | Grey group 200C | 100 | 14.75 |
| 40 | Grey group 200C | 100 | 20.0 |

cause of erratic germination in okra (Demir, 7). The hard seed coat of okra interferes with water uptake and is a major physiological constraint to uniform stand establishment and performance (Standifer *et al.*, 22).

Some studies have shown an interrelation between seed moisture and hardseededness. Ellis *et al.* (10) reported little to no hardseededness at 13% but a considerable amount at 4-6% moisture content. Generally, in species that exhibit physical dormancy, hardseededness develops during maturation drying of the seed, with seed coats becoming impermeable at moisture contents anywhere from 15 (Mai-Hong *et al.*, 18) to 54% (Cabrera *et al.*, 4). Our study also revealed that there were no hardness in fresh seeds (Fig. 1) having moisture content of 72.3% at 10 DAA, 60.2% at 17 DAA and 48.9% at 24 DAA. With the advancement of seed development there was a drastic reduction in moisture content and the occurrence of hard seeds were observed. Hard seeds were 11.7 and 14.7% at 32 and 36 DAA, when seed moisture content was 18 and 12.6% respectively. Maximum hard seeds (20%) were found when moisture content was (7.9%) at 40 days after anthesis in fresh developing seeds. Similarly, no hard seeds were found in rapidly dried seed till 24 DAA but with further drastic reduction in moisture content, the occurrence of hard seeds were observed. Hard seeds were 23.7 and 25.5% at 32 and 36 DAA, when seed moisture content was 9.3 and 9.2% respectively in rapidly dried seeds. Maximum hard seeds (26%) were found when seed moisture content was (7.8%) at 40 days after anthesis. At 50 DAA when seed moisture content had dropped to 10 and 52% of seeds tested were hard seeded in okra cv. Akkoy (Demir, 8).

In fresh okra seeds, maximum germination of 64.7% (Fig. 1) occurred around 36 days after anthesis when physiological maturity and maximum seed dry weight (68.4 g) were attained (Fig. 2b). In rapidly dried seeds, maximum germination (61.5%) was attained at 36 days after anthesis. In fresh seeds, this stage of seed development was marked by drastic reductions in seed leachate conductivity (Fig. 2c) and seed moisture content (Fig. 2a). Pandita and Nagarajan (20) also reported rapid declines in chlorophyll content, leachate conductivity and seed water content with the advancement of onion seed development, however seed dry weight and germination increased. Changes in seed quality during development on the mother plant were also investigated by Demir (6) in okra cultivar. Mass maturity (the end of seed filling an period) occurred 31 days after anthesis at which seed moisture content was around 71%. Maximum seed quality assessed by germination, emergence and rapid ageing test was recorded 52 days after anthesis. Maturation drying reduced seed moisture content to 12% at harvest. A study conducted on *Malva parviflora* indicated that seed moisture content decreased as they developed, whereas fresh and dry weights increased to peak at 12-15 and 21 days after flowering, respectively. Therefore, physiological maturity occurred at 21 days after flowering, when seed moisture content was 16-21%. Seeds were capable of germinating early in development, reaching a maximum of 63% at 9 days after flowering, but germination declined as development continued, presumably due to the imposition of physiological dormancy. Physical dormancy developed at or after physiological maturity, once seed moisture content declined below 20%. Seeds were able to tolerate

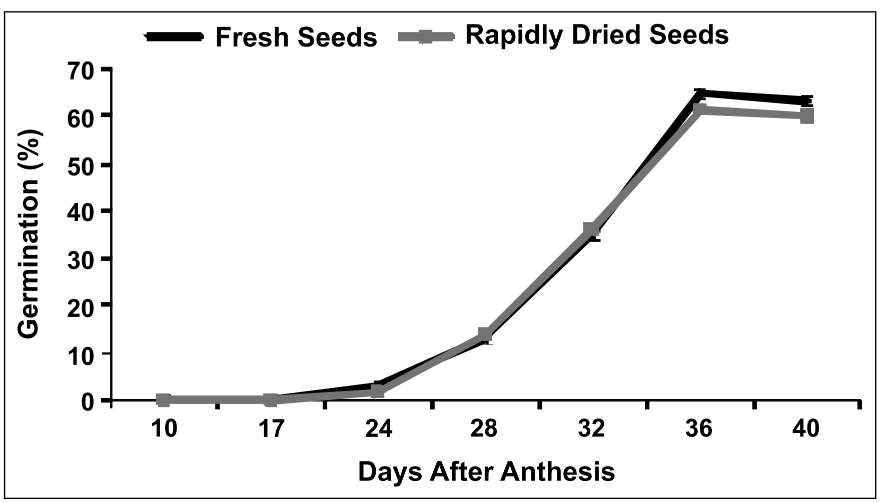


**Fig. 1.** Changes in germination (%) in fresh and rapidly dried seeds of okra cv. Pusa A-4 with increasing days after anthesis.

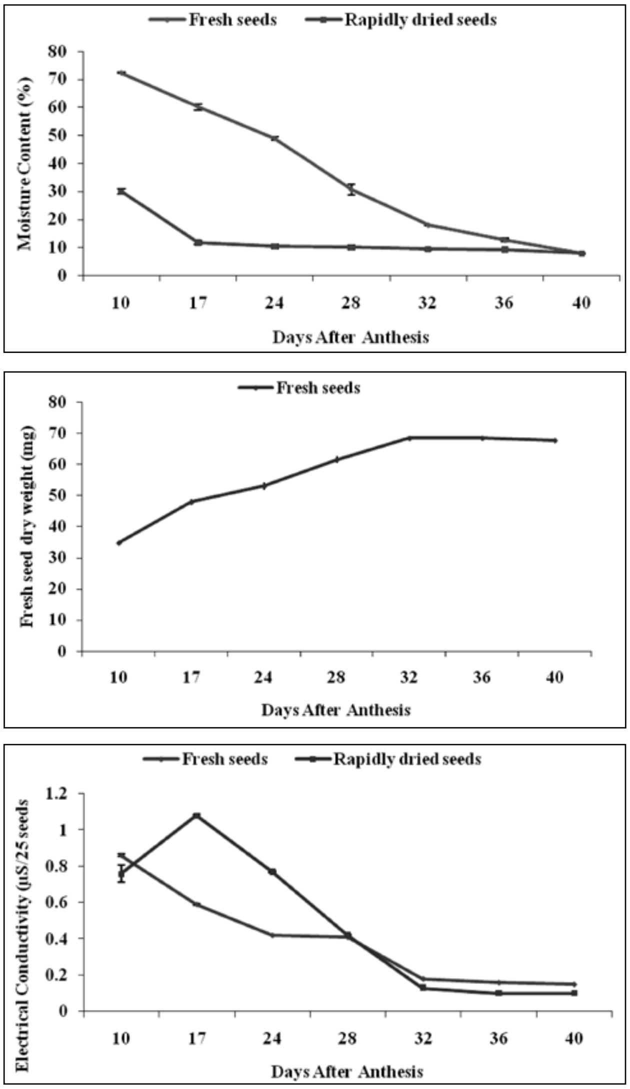


**Fig. 2.** Changes in (a) seed moisture content. (b) fresh seed dry weight and (c) seed leachate electrical conductivity in fresh and rapidly dried seeds of okra cv. Pusa A-4 with increasing days after anthesis.

desiccation from 18 days after flowering; desiccation hastened development of physical dormancy and improved germination (Michael *et al.*, 19). Ellis *et al.* (9) reported that peak maturity, *i.e.*, maximum seed quality following harvest and rapid artificial drying, was achieved in six legumes species (faba bean, chickpea, white lupin, soybean, lentil and pea) once maturation drying had reduced the moisture content of the seeds to 45-50%. In pea, faba bean and soybean there was a substantial decline in viability and an increase in seedling abnormalities when harvest was delayed beyond the optimal moisture content for harvest. Harrington (12) suggested that physiological maturity is also the developmental stage at which seeds achieve maximum viability and vigour since nutrients are no longer entering the seed from the plant, and thereafter seeds begin to age. This implies that physiological maturity is the most suitable stage at which seed should be harvested, provided they can tolerate harvest and rapid drying. It was found in *Aesculus hippocastanum*, mean fresh and dry weights increased for both the axes and the whole seed up to the time of peak seed fall at 135 days after anthesis with no cessation before fruit abscission. Decrease in seed moisture content during development was accompanied by increase in desiccation tolerance and in germinabiltiy, both reaching their maximum at the time of peak seed fall (Tompsett and Pritchard, 24).

Changes in cellular membranes play a key role in the acquisition of desiccation tolerance by developing seeds (Le-Page-Degivry and Garello, 17). The ability to maintain membrane integrity and stability on drying is essential for seed axes to tolerate desiccation. Desiccation disrupts cellular membranes during the early intolerant stage of seed development. This induces massive leaching of ions, phosphate, sugars and soluble proteins during subsequent rehydration (Adams *et al.*, 1). We also observed that membrane leakage decreased linearly as developing okra seeds matured from 32 DAA (Fig. 2c). The conductivity of seed leachates from both rapidly dried and fresh developing seeds of okra were low at physiological (36 DAA) and harvest maturity (40 DAA). The electrolyte conductivity of the seed leachates offered a quantitative measure of desiccation tolerance. Sun and Leopold (23) reported a similar linear decrease in membrane disruption in excised axes of soybean as they matured from 30 to 55 DAA. Since poor quality seed often leak more exudates than good quality seeds during the first hours of imbibitions, the conductivity test has become an important approach to monitoring seed quality in large seeded legume (Hampton, 11). Powell (21) emphasized that the degree of seed leakage during imbibitions is influenced by stage of seed maturation, degree of seed ageing and incidence of seed damage.

Several metabolic changes occur in seeds either just before or during drying. These changes involve the appearance of two types of products, namely carbohydrates (oligosaccharides and sugars) and proteins. Specific proteins are perceived to have functional significance with respect to protection of seed tissues against the rigours of desiccation (Bewley and Black, 3). The present investigation also indicated a sharp increase in sugars towards physiological maturity both fresh and rapidly dried developing okra seeds. Maximum total soluble sugars content was recorded at 36 DAA. No sharp increase

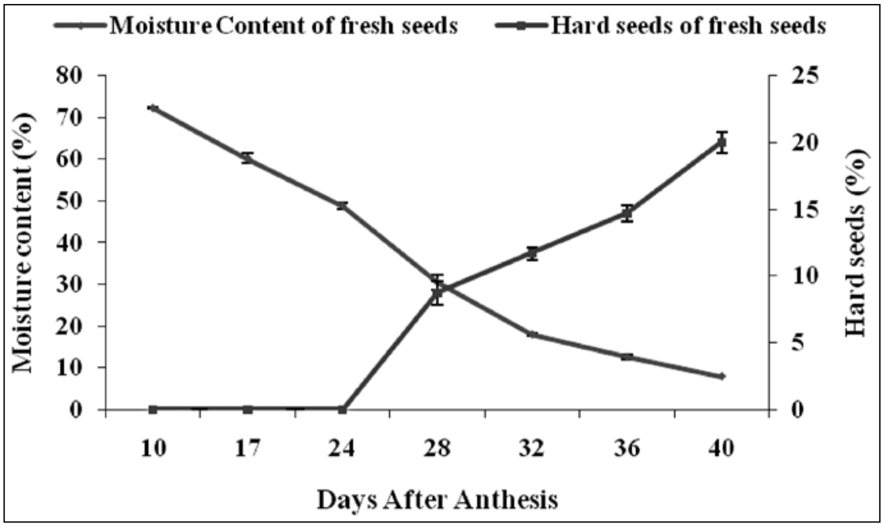


**Fig. 3.** Relationship between seed moisture content and occurrence of hard seeds in okra.

in reducing sugar content during seed development was observed, however, non-reducing sugar content of fresh and rapidly dried seeds increased after 28 DAA. Maximum non-reducing sugars (143.4 mg/g DW) were obtained at 36 DAA in rapidly dried seeds at which maximum seed germination was observed (Fig. 4b). In onion, Pandita and Nagarajan (20) reported maximum germination at 42 days after anthesis in shade-dried seeds and at this stage concentration of non-reducing sugars increased many fold. Decline in the number of reducing sugars (e.g. glucose, fructose, galactose and mannose) and increases in

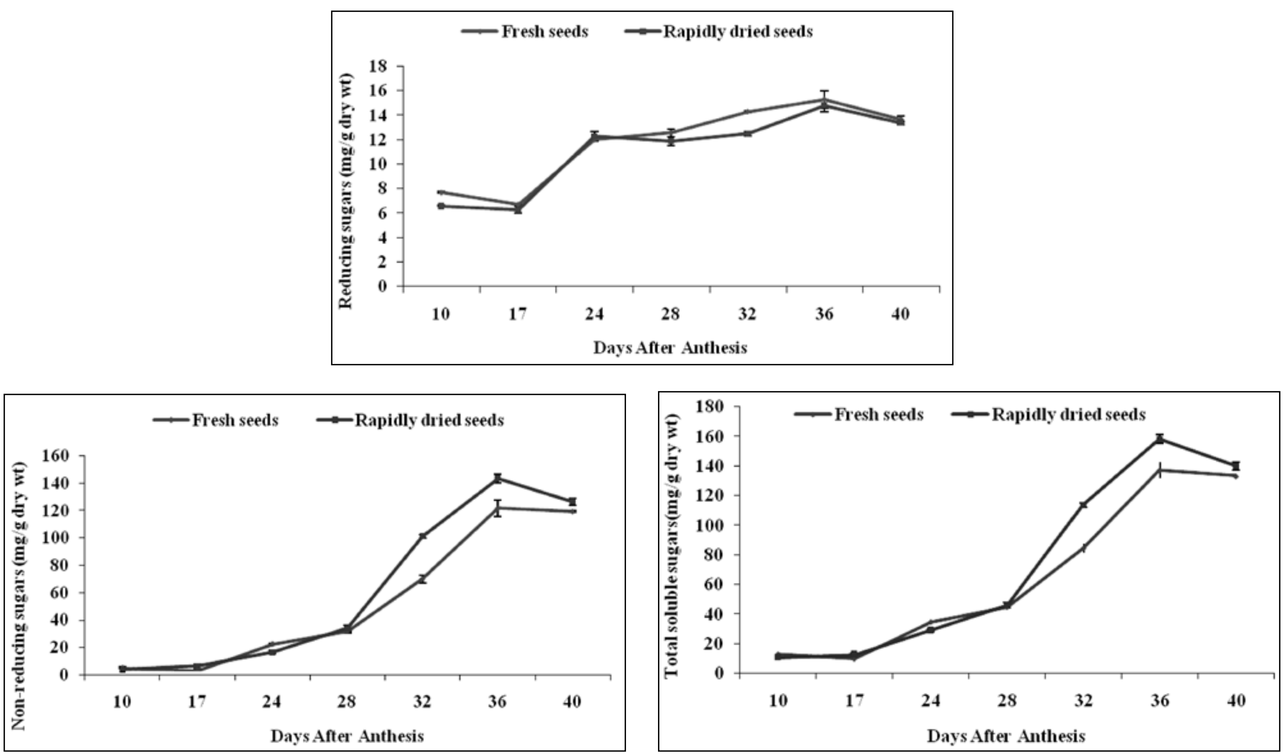


**Fig. 4.** Changes in (a) reducing sugars, (b) non-reducing sugars and (c) total soluble sugars, in fresh and rapidly dried seeds of okra cv. Pusa A-4 with increasing days after anthesis.

sucrose and oligosaccharides such as raffinose and stachyose have been reported during the acquisition of desiccation tolerance in *Brassica campestris* L. (Leprince *et al.*, 16), soybean (Sun *et al.*, 23), and maize (Chen and Burris, 5). Many species acquire the ability to germinate only when the seed switches from a developmental to a terminative mode during maturation drying. Although this can be induced by removing the seed from the plant during development, seeds are not tolerant of drying at every stage during their development and will also undergo a transition from desiccation intolerant to desiccation tolerant (Kermode and Bewley, 14).

In conclusion, harvesting the okra cv. Pusa A-4 seed crop as early as 36 DAA, produced seeds with equivalent quality to that of seeds harvested at full maturity. This stage could easily be identified by the sudden decrease in seed water content and seed leachate conductivity. This has practical applicability in okra seed production as harvesting before full maturity can be employed in the event of adverse weather conditions and to eliminate shattering losses.

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