

## Seed conditioning as an effective method to alleviate imbibitional injury in some leguminous crop seeds

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**ABSTRACT** Seeds in some leguminous crops *viz.* broadbean, chickpea (Brown), chickpea (Cream), cowpea, greengram, lentil and soybean conditioned to 9, 6 and 4 % moisture contents were subjected to standard germination test. Results showed that cowpea and broadbean seeds were most susceptible to imbibitional injury. Soaking of these seeds in water for 16-20 hr prior to standard germination test resulted in a greater proportion of seeds failing to germinate, the degree of imbibitional injury being maximum in cowpea followed by broadbean. Seed conditioning (Slow rehydration) of these seeds to 17-21% moisture content was effective to mitigate the imbibitional damage. It is evident from the above study that the damage to the seeds following desiccation is more likely to be a consequence of imbibitional damage to which the seeds are more susceptible rather than a consequence of desiccation per se. The imbibitional damage can be mitigated to a large extent by slow conditioning of these legume seeds.

**Key words:** Seed conditioning, imbibitional injury, legume seeds, germination

One of the pre-requisites for long-term conservation of majority of crop species in genebanks is to dry the seeds to low moisture contents. Prior germination tests to long-term conservation of genetic resources is aim to assure the good seed quality in each accession and/or to detect any loss in seed viability. Most of the genebanks adopt the procedures recommended by International Board of Plant Genetic Resources (IBPGR) [1]; where seeds are dried to 5±1% moisture content (mc). Seeds during long-term conservation are periodically removed for monitoring their viability *i.e.* to assess the status of seed accessions for rejuvenation before their germinability is lost forever. There were reports suggestive of imbibitional damage occurring in some leguminous seeds with low moisture contents. Tittel [2] had shown that soybean and frenchbean seed lots desiccated to 4% mc showed decrease germination. Ellis *et al.* [3] had also reported that there was a decrease in the

proportion of cowpea seed germinating normally, if dried from 12 to 4.4% mc. Seed conditioning of very dry seeds resulted in germinating them normally. An attempt was made to investigate the influence of desiccation of some legume crop seeds to lower mcs (6%) on their germinability and devise some effective methods to overcome the imbibitional injury, thereby avoiding the artifacts in the estimation of viability (Monitoring of seed viability) during long-term conservation under genebank environments.

### MATERIALS AND METHODS

Seeds of cultivated varieties of broadbean (*Vicia faba* var. Local), chickpea-brown (*Cicer arietinum* var. Pusa 267), chickpea-cream (*Cicer arietinum* var. Pusa 209), cowpea (*Vigna unguiculata* var. 240), greengram (*Vigna radiata* var. PS 16), lentil (*Lens culinaris* var 1096) and soybean (*Glycine max* var. Pusa 22) were dried at 15°C and 15% RH to

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bring down to chosen moisture contents of 9 and 6%. Further reduction in mc to 4% was achieved by freeze drying the samples using lyophilizer (Virtis, USA). The initial moisture content of these seed lots was determined by the constant temperature oven method of International Seed Testing Association (ISTA) [4]. Each seed lot conditioned to a chosen mc was divided into four sublots of having 200 seeds each. Each subplot was subjected to four different types of seed conditioning treatments: a. untreated (Control), b. slowly imbibed between two layers of moist filter papers for 3 hr, c. humidified over saturated water vapours in a desiccator at 20°C until the seed attained mcs (between 18 to 20%) and d. immersed the seeds in water for 16 hr at 20°C. Seeds were surface dried before use, after removing them from water. These sub-lots of seeds were tested for standard germination following ISTA rules [4], with some modifications. One hundred and fifty seeds, in three replications of 50 seeds each, were tested using rolled towel paper method at 25°C and evaluated on 07 day from seed planting.

## RESULTS AND DISCUSSIONS

The moisture content of the seeds (Initial) in various crops ranged from 9.5-10.5%. The number of seeds that failed to germinate normally, when tested by the standard germination test (Without conditioning) increased significantly in desiccated cowpea and broadbean. The extent of imbibition injury being greater at 4% than at 6% mc. Slow and controlled hydration is essential as the first step in the reactivation of metabolic processes in the dry seed leading to germination and growth [5]. Seed conditioning (Re-humidification) of both the seed lots by moist filter paper impregnation and over water saturated atmosphere at 20°C could significantly mitigate the imbibitional damage as evident from the present data on seed germination (Table 1). Alleviation the effects of soaking injury as a result of increase in the mc of seeds before imbibition is related to the reduced binding energy of the water molecules and the appearance of respiratory activity [6-8]. During seed conditioning (Re-humidification), the dry membrane of the cells is hydrated slowly with

the hydrophylic components of the membrane getting hydrated first resulting in rearrangement of the membrane components to form a functional bilayer membrane with selective permeability [9-11]. On the contrary, if there is in rush of large amount of water, excess of water molecules not used up in the dry membrane hydration process pass through non-functional membranes. This excess water encountering stored macromolecules (like, proteins and carbohydrates) in the cotyledons and axis hydrates them thus resulting in their swelling [12-13]. This eventually leads to soaking injury due to the rupture of the irregular membrane configuration in the dry seeds [14-15]. The direct soaking of seeds of all crops except brown chickpea in water resulted in significant decline in germination percentage, even at 9% mc establishing their susceptibility to imbibitional injury. Chickpea, greengram and lentil seeds registered higher germination values without any pre-treatment despite their low mcs and therefore do not require any seed conditioning (Re-humidification) treatment prior to their standard germination test. Powell and co-workers [16] had demonstrated that testa pigmentation is a significant determinant of the rate of water uptake during imbibition in the cultivars of common bean, cowpea, chickpea and longbean. In soybean seeds, there was no significant decrease in germination when seeds of lower moistures were planted for standard germination test but seed conditioning (Re-humidification) marginally improved their germination (Table 1). On the contrary, Obendorf & Hobbs [17] and Tittel [2] had clearly demonstrated the role of re-humidification in reducing the damage caused by rapid uptake of water by dry soybean seeds. The decrease in the proportion of normal seedlings in cowpea and broadbean which occurred as a result of rapid imbibition is consistent with that observed in cowpea cultivars [3] and broadbean [18]. Several reports have shown that the incidence of imbibitional damage was closely associated with the rate of imbibition of various cultivars [19-20]. Humidification prior to testing for germination or field sowing is reported to be beneficial for seeds of many grain legume species for example lima bean [21], soybean [2, 17], frenchbean [22] and broadbean

Table 1. Effect of seed conditioning on seed germination (%) in some legume crop seeds

Treatment	Moisture content (%)	Crop						
		Broad-bean	Chickpea (Brown)	Chickpea (Cream)	Cowpea	Greengram	Lentil	Soybean
Control	9	81.00	99.00	99.00	89.00	99.00	96.00	93.00
	6	65.00	98.00	99.00	57.00	100.00	95.00	91.00
	4	31.00	98.00	96.00	41.00	99.00	92.00	92.00
MFP	9	93.00	100.00	97.00	90.00	99.00	96.00	94.00
	6	92.00	99.00	97.00	59.00	99.00	96.00	96.00
	4	73.00	94.00	99.00	49.00	100.00	94.00	93.00
WSA	9	92.00	99.00	96.00	88.00	99.00	97.00	93.00
	6	92.00	99.00	99.00	85.00	98.00	96.00	98.00
	4	80.00	98.00	97.00	77.00	97.00	92.00	96.00
DI	9	13.00	100.00	91.00	25.00	96.00	97.00	84.00
	6	13.00	95.00	66.00	5.00	92.00	80.00	45.00
	4	3.00	94.00	36.00	0.00	49.00	80.00	16.00

LSD  $\geq$  5%: 6.226; Where, MFP: Moist filter paper; WSA: water saturated atmosphere; DI: direct immersion

[18]. Seed germination results showed that damage to the above leguminous seeds may result from low initial mcs and rapid imbibitions. These factors depend on the seed coat characteristics and may not be true for different cultivars of the same species. Recently, Nakayama and Komatsu [23] had demonstrated that between two yellow seeded soybean varieties, the one in which the seed coat adhered loosely to the embryo was more susceptible to imbibitional injury. The substantial damage that occurred in cowpea and broadbean (Present study) could be attributed to such seed coat characteristic. On the other hand, Chachilis and Smith [24] reported that the rapid imbibition in some soybean cultivars was due to the presence of wide open pores on the seed coats, whereas these were absent in slow imbibing cultivars. In the present study, the different crop seed lots conditioned to 6 and 4% mcs had achieved a moisture content between 16.86 to 21.87% when placed in a water saturated atmosphere at 20°C for a period of 4

days, the two exceptions being broadbean and greengram, which could attain mcs between 9.8 to 14.8% (Table 2). Even at these moistures the imbibitional damage could be greatly reduced (Table 1). It is therefore suggested that these seeds be conditioned (Re-humidified) to avoid loss in germination especially for the seed stocks labeled for field sowing or when they are removed from the genebanks for viability monitoring so that a true estimate of viability is available. More care needs to be taken particularly for cowpea and broadbean seeds before subjecting to standard germination test, whereas seeds of soybean, greengram, lentil and chickpea (Cream colour seed coat) need to be subjected to this treatment whenever studies demand their direct soaking in water such as pre-conditioning for quick viability test using triphenyl tetrazolium chloride, soaking for recording conductivity measurements or studies involving water absorption rates.

Table 2. Moisture content attained by the different seed lots by seed conditioning (Re-humidification) at 20°C

Crop	Moisture content (%)		
	Initial	Final	
		WSA	MFP
Broadbean	9	14.74	26.37
	6	11.10	16.81
	4	9.80	13.90
Chickpea (Brown)	9	20.77	35.79
	6	16.86	31.65
	4	17.59	32.00
Chickpea (Cream)	9	19.19	37.39
	6	19.19	38.17
	4	17.94	37.17
Cowpea	9	21.13	40.64
	6	20.32	39.13
	4	20.62	41.35
Greengram	9	21.87	41.93
	6	14.80	38.12
	4	13.93	33.67
Lentil	9	21.51	46.84
	6	20.21	43.19
	4	18.00	36.76
Soybean	9	21.57	44.79
	6	21.13	38.75
	4	18.95	37.87

Where, WSA: Water saturated atmosphere, MFP: between moist filter papers

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