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Quality improvement of aged cabbage (*Brassica oleracea* var. *capitata*) seeds using chlorophyll fluorescence sensor

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ABSTRACT

Fourteen old seeds of cabbage (*Brassica oleracea* var. *capitata*) were sorted on the basis of chlorophyll fluorescence (CF) signal. Seed fractions of 54.3% and 45.7% with low CF and high CF, respectively, were obtained. Unsorted seeds were considered as controls. The effects of CF sensor based sorting on quality parameters of aged cabbage seeds were examined. It was observed that the seeds with low CF resulted in significantly higher germination, vigour and germination rate over unsorted fraction. However, no significant differences were noticed in ethanol produced (μg/l) at 40 °C in unsorted, low and high CF seeds. Significantly less numbers of hard seeds were noticed in seeds with low CF, whereas numbers of abnormal seedlings and dead seeds were found at par with unsorted seeds. Numerically higher values over unsorted seeds for fresh weight, dry weight, root and total seedlings length were found in seed fraction with low CF. The sorting improved the germination of aged cabbage seeds by 16%. Therefore, this method of quality up-gradation could be used to obtain a better quality seeds even from the lots which otherwise become substandard during storage. The technique could also be commercially exploited for sorting seeds and upgrading the quality of all those crop seeds where chlorophyll is present in the seed coat and persist during storage.

1. Introduction

More than hundred types of vegetables can be grown over an area of 7.98 million hectare in India due to its unique and diverse climate. In addition, India is the second largest producers of vegetables with 32.31% share of global vegetable production. However, the pressure to produce more is mounting with increasing population and depleting agricultural land, water and other resources. In last one decade, there has been considerable progress in the productivity of vegetables which has presently increased to 16.7 tonnes per hectare. But with this, only 145 g per capita per day vegetables are available to its citizens against recommended requirements of 280 g. To meet this demand, vegetable production has to be increased by 2.5% annually. Hence, to ensure food and nutritional security from the limited area and scarce resources we have to increase the yield. Cabbage (*Brassica oleracea* var. *capitata*) amongst all brassicas is most important vegetable, especially in the temperate zones of the world. This being one of the most preferred winters vegetable, has the potential to contribute significantly towards the projected demand of vegetables. A total of 8.53 mt of cabbage was produced during 2013 from an area of 0.372 mha which constitutes 5.26% of total vegetable production in India (Anonymous, 2013). The seed quality of improved genotypes is the most critical for increasing the production and productivity of cabbage up to 20% and it can be further raised up to 45% with efficient management of other inputs.

Vigour and viability are the measure of quality of any seed lot. Seed vigour is maximum at physiological maturity and is important for obtaining uniform, healthy and vigourous crop stand. The vigour and viability of seeds start decreasing soon after the onset of seed drying on mother plant itself (Olasoji et al., 2012). Moreover, the seeds gradually lose their viability and vigour in storage depending on conditions, time and genotype (Balesevic-Tubic et al., 2010). Mitochondria are the primary sites of deterioration in storage (Bewley and Black, 1994) and known to have relation with premature harvest of seeds. The energy required for metabolic

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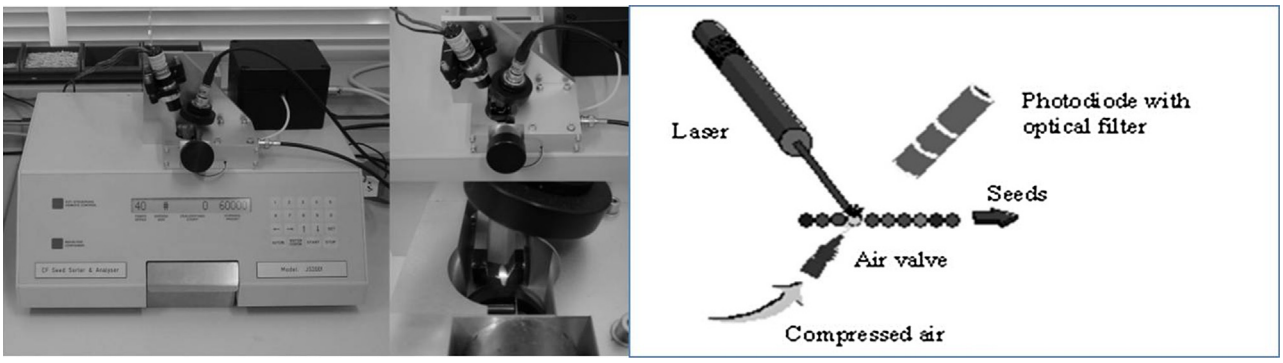

Fig. 1. Setup of the JS 2001 Seed Sorter equipment and schematic representation used for chlorophyll fluorescence measurements. (LED: light emitting diode, λ_{max} = 650 nm, full width half maximum (FWHM) = 22 nm; LED-PS: LED power supply; filter 656: interference filter, λ_{max} = 656 nm, FWHM = 10 nm; beam splitter: 50% transmission, 50% reflection; lens: f = 15 mm, Ø = 15 mm; filter 730: interference filter, λ_{max} = 730 nm, FWHM = 10 nm; photodiode: Ø = 11.3 mm).

processes is met through anaerobic respiration when mitochondria start non-functioning due to damage in storage, which could stimulate ethanol production. Ethanol in an imbibing seed is produced by conversion of pyruvate in presence of the regulatory enzyme, pyruvate decarboxylase. Loss of mitochondrial membrane integrity due to seed deterioration is expected to enhance ethanol production and estimation of that could give an estimate of seed viability as well as vigour. Seed deterioration has a direct correlation with ethanol production (Rutzke et al., 2008). Several alternatives to standard germination test like electrical conductivity, accelerated ageing, potassium leakage and controlled deterioration are available for distinguishing quality and vigour of stored seed lots in diverse crops, but all are destructive. Thereby, need for the non-destructive method of separating the aged seeds with the assessment of physical and physiological condition of seeds was realized and different workers tried various techniques to ease the pressure on seed industry. Chlorophyll content in seed coat is the measure of seed maturity and the fluorescence parameters can be used to evaluate chlorophyll degradation after the physiological maturity to assess the quality (Jalink et al., 1998). However, no information exists till date on use of this rapid and non destructive technique in naturally aged seeds. Therefore, the present investigation was carried out to assess the possibility using chlorophyll fluorescence for quality up-gradation of cabbage seeds stored for long.

2. Materials and methods

Cabbage (*Brassica oleracea* var. *capitata*) variety B253 seeds with 96% germination, having 8.0% seed moisture were stored at 5 °C in hermetically sealed container during April 1997–April 2011 at Plant Research International, Wageningen. The same seeds were tested for viability and moisture content as per ISTA (Anonymous, 2010) and used for the investigations during April, 2011. The seeds were sorted on the basis of chlorophyll fluorescence (CF) signal into two fractions i.e. seeds with high and low CF signals with help of the JS 2001 Seed Sorter equipment, setup of which is depicted in Fig. 1 (Jalink et al., 1998).

JS 2001 Seed Sorter equipment takes into account the surface area of seed that is illuminated by the light, the structure of the seed coat, the spectral sensitivity of the filter or photodiode combination and the solid angle of the lens system. Further, the experimental conditions that predominantly determine the magnitude of the chlorophyll fluorescence signal are the spectral distribution and intensity of the excitation light (Nobel, 1970).

For sorting on the basis of chlorophyll fluorescence signal one thousand cabbage seeds were fed thrice in the hopper of the JS 2001 Seed Sorter, sequentially. Thus, the seed samples of each replication were sorted into two fractions, one with low and the other with high chlorophyll fluorescence seeds, using the noise levels of 40 pA and sorting level of above 176 pA (Fig. 2). Thus, the three seed lots/fractions viz.; control (original seed lot/un-sorted), the low CF seed fraction with signal lower than 176 pA but above 40 pA and the high CF seed fraction with signal higher than 176 pA constituted the experimental material. These three fractions were subjected to test the germination, vigour and assessment of ethanol production.

Three replications of hundred seeds from each fraction were used to test the percent germination and vigour parameters. Twenty-five seeds were put for germination in 4 different petri plates to constitute one replication. Germination was tested as per ISTA (Anonymous, 2010) by placing the petri plates in an incubation chamber at 20 °C for 10 days. The numbers of seeds germinated were counted daily till 10th day of putting. On the final count day, seedlings were categorized into normal, abnormal seedling, hard and dead seeds. The seeds absorbed water but did not germinate (remained fresh) were also counted in numbers of hard seeds. The percentage of normal seedlings was used to calculate standard germination. Five normal seedlings were taken at random from each replication for measuring root, shoot and total length (cm) of each seedling. The same seedlings were weighed for fresh weight and put for drying in a hot air oven maintained at 70 ± 1 °C for 48 h. Seedling dry weight was taken after cooling them. The vigour indices were computed (Yadav et al., 2013) adopting the following formula; Vigour index I = Germination (%) × Total seedling

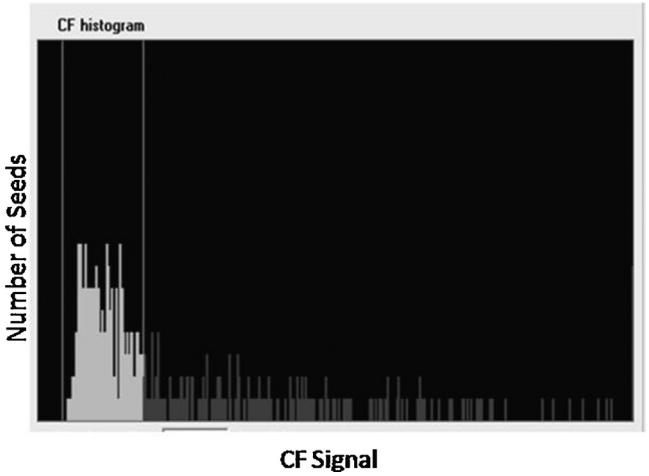

Fig. 2. Histogram showing number of seeds in fractions of low (Left) and high (Right) chlorophyll fluorescence (CF).