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Research article

An analysis of the development of cauliflower seed as a model to improve the molecular mechanism of abiotic stress tolerance in cauliflower artificial seeds

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ABSTRACT

The development stages of conventional cauliflower seeds were studied and the accumulation of dehydrin proteins through the maturation stages was investigated with the aim of identifying methods to improve the viability of artificial seeds of cauliflower. While carbohydrate, ash and lipids increased throughout the development of cauliflower traditional seeds, proteins increased with the development of seed and reached the maximum level after 75 days of pollination, however, the level of protein started to decrease after that. A significant increase in the accumulation of small size dehydrin proteins (12, 17, 26 KDa) was observed during the development of cauliflower seeds. Several experiments were conducted in order to increase the accumulation of important dehydrin proteins in cauliflower microshoots (artificial seeds). Mannitol and ABA (Absisic acid) increased the accumulation of dehydrins in cauliflower microshoots while cold acclimation did not have a significant impact on the accumulation of these proteins. Molybdenum treatments had a negative impact on dehydrin accumulation. Dehydrins have an important role in the drought tolerance of seeds and, therefore, the current research helps to improve the accumulation of these proteins in cauliflower artificial seeds. This in turns improves the quality of these artificial seeds. The current results suggest that dehydrins do not play an important role in cold tolerance of cauliflower artificial seeds. This study could have an important role in improving the understanding of the molecular mechanism of abiotic stress tolerance in plants.

1. Introduction

An effective protocol for cauliflower micropropagation was designed by Kieffer et al. (2001) and optimized by Rihan et al. (2011b), (2012)and enables the production of thousands of microshoots per cauliflower curd and provides an optimal system for the analysis of the physiological and molecular responses of cauliflower to various types of abiotic stresses. Moreover, these microshoots can be used for artificial seed production, which are required to show a high level of abiotic stress tolerance in order to be a cost effective method for cauliflower propagation. Insights into how to make artificial seeds more successful can come from studies of natural seed development.

Natural seed development involves a series of changes from ovule fertilization to seed maturation that is genetically controlled. This development comprises a series of morphological, physiological and biochemical changes occurring from ovule fertilization to the time when seeds become physiologically independent of the parent plant (Delouche, 1971). Fellows et al. (1979) found that the physiological maturity of orthodox-seeds generally occurs when the moisture content declines to about 50–60% and that at this stage, the seeds exhibit the highest level of viability, vigour and dry weight. Seed maturity is normally accompanied by noticeable changes in seed and fruit colouration (Nkang, 2002). Seed development can be divided into four different stages: embryo patterning, embryo growth, seed filling and seed desiccation (Fei et al., 2007). After the completion of embryo growth, major increases include accumulation of seed storage products such as protein, oil and carbohydrate. The seed filling stage is followed by the desiccation stage during which seeds acquire drought tolerance (Bewley and Black, 1994). Coelho and Benedito (2008) divided the

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development of seeds into three main stages according to the dry components accumulation: the first stage is characterised by a relatively slow mass accumulation during embryogenesis. The second stage is the maturation stage, characterised by a continuous and high increase in dry matter. This stage ends by reaching the maximum dry matter content at physiological maturity. Seed dehydration is the third phase of the seed development. This stage is characterised by biological mechanisms leading to embryo desiccation resistance.

Late Embryogenesis Abundant (LEA) proteins were first described in wheat and cotton (Goyal et al., 2005) and are synthesised in abundance during seed development and can comprise up to 4% of cellular proteins (Roberts et al., 1993). LEA proteins have been linked to the embryo capability for withstanding dehydration although the mechanism of action is still not clear (Coelho and Benedito, 2008). One of the mechanisms proposed with regards to LEA functions was that these proteins might act as protectors of macro-molecular and/or cellular structures during water deficit since they preferentially network with the available water molecules and deliver a hydration shell to protect the "integrity" and function of these macro-molecules (Hoekstra et al., 2001; Garay-Arroyo et al., 2000). LEA proteins also, together with oligosaccharides and perhaps small heat shock proteins, (sHSPs), participate in the formation of the "glassy state" of the seed and its stabilization in the dehydrated state (Kalemba and Pukacka, 2008). LEA proteins increase hydrogen bonding and, thereby, the average strength of the amorphous matrix and the glass transition temperature (Wolkers et al., 2001).

LEA protein genes have been described in different plant species and six different groups of these proteins have been characterised according to their expression patterns and gene sequence. The main classes are group 1, 2 and 3 (Wise, 2003; Bray, 1993). Group 1 consists of LEA proteinsthat are found only in plants and are unstructured proteins in solution. This group of proteins has a preserved 20-residue amino acid motif, most often in one copy (Goyal et al., 2005). Group 2 LEA proteins, which are known as dehydrins, are mainly found in plants (Close et al., 1989). This group of proteins is classified in three sequence motifs described as the K-domain (lysine-rich), the Y-domain (DEYGNP) and the S-segment (polyserine stutter) (Kalemba and Pukacka, 2008). However, the K-domain, which contains the consensus amino acid sequence EKKGIMDKIKELPG, is the only segment present in all types of dehydrins (Close, 1997). Based on the existence and combinations of segments, dehydrins are categorized into the classes Y_nSK_2, K_n, K_nS, SK_n and Y_2K_n (Kalemba and Pukacka, 2008). Although dehydrins show some α-helical content, they are considered to be unstructured proteins (Lisse et al., 1996; Ceccardi et al., 1994). Group 3 LEA proteins are defined by a repeated 11-mer amino acid motif. The consensus sequence of this motif has been widely described as ΦΦE/QXΦKE/QKΦXE/D/Q (where Φ characterizes a hydrophobic residue). This group of proteins has been identified in the homologues of organisms other than plants (Dure, 2001).

It is widely accepted that both freezing stress and drought cause desiccation of the plant cell protoplasm (Steponkus et al., 1980). Freezing stress causes ice formation in the intercellular spaces and, since ice has a lower osmotic potential than water, water moves from inside the cells to the ice in the intercellular space resulting in desiccation of the cytosol (Thomashow, 1999). Several studies suggest that plants have similar approaches to desiccation resistance regardless of whether the desiccation is caused by drought or freezing stress and several genes have been found to respond to both drought and freezing stresses (Shinozaki and Yamaguchi-Shinozaki, 2000). It also seems that there is a physiological cross adaptation where the exposure to one stress can improve the tolerance to other stresses (Parmentier-Line et al., 2002) where drought can predispose plants to cold tolerance (Anisko and Lindstrom, 1996) and vice-versa (Levitt, 1960). It has been demonstrated that groups of dehydrin proteins are produced by conditions which have a dehydrative constituent such as drought, salinity, cold and ABA (Close, 1997). The current study aimed to investigate the developmental stages of cauliflower seeds in terms of the accumulation of seed reserve compounds (lipids, carbohydrates, minerals and proteins) and in terms of the changes in the amount of dehydrin protein which occurs. It was an aim to try to improve artificial seeds by mimicking processes occurring in conventional cauliflower seeds. Moreover, it aimed to investigate the effect of certain treatments such as Mannitol, and Molybdenum (MO) treatments which were reported to have a positive effect on cauliflower artificial seed cold tolerance (Rihan et al., 2014; Rihan, 2014), on the accumulation of dehydrin proteins in cauliflower microshoots and, therefore, to find out whether dehydrin proteins play any part in the cold tolerance of cauliflower artificial seeds (microshoots).

2. Material and methods

2.1. Plant materials

Twenty young cauliflower plants (cv Medallion, Bred by Bejo (Holland). Sold in the UK by Elsoms seeds) were obtained from a field in Cornwall, courtesy of Simmonds & Sons Ltd, and replanted in large pots in the Skarden Garden greenhouse at the University of Plymouth. The plants were grown according to good commercial practice and fertilized appropriately and given crop protection against aphid and leaf disease as necessary. Five plants of the cultivar Fleet were cultured between the Medallion plants to facillitate sufficient open pollination between the two varieties in view of the self-incompatibility nature of cauliflower and the necessity for external pollinators. When the plants started flowering, they were covered with fleece bags for 8 days to ensure that there was a significant amount of flowers available for fertilization. The bags were then removed for a week to allow open pollination between cauliflower plants. The plants were then re-covered to stop further pollination (Fig. 1). Giving a limited period for open pollination helped to increase the level of synchronization in cauliflower seed maturation. A month later, the first sample of cauliflower seeds was collected. Subsequently, seed samples were collected five times at 15 day intervals (Fig. 2) (It was difficult to collect any more seeds after that since they had become very dry and the seeds started falling from the plants). Samples were placed in nylon bags and transferred quickly to a fridge at 4 °C and the subsequent analyses were done on the day of the sample collection or on the following day. Determination of moisture content.

Three replicates, each consisting of 5 g seeds, were used from each seed sample. Seeds were dried to a constant weight in an oven at 130 °C. The moisture level was calculated as:

$$\%\ \text{Moisture} = \frac{W1 - W2}{W1} * 100$$

W_1: Seed fresh weight.
W_2: Seed dry weight.

2.2. Determination of lipid content (Rapid Soxhlet Extraction)

Rapid Soxhlet Extraction methods were used to determine the lipid content of cauliflower seeds following the procedures