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Research Note

The interaction between salinity stress and seed ageing during germination of *Brassica napus* seeds

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Abstract

A study was designed to examine the effects of NaCl, high temperature, high relative humidity and storage on the germination of *Brassica napus* seeds, both when freshly harvested and after natural and accelerated ageing. After exposure to a combination of stressful conditions, the intensity of lipid peroxidation in the seeds, seedling shoot and roots, and seed germination were measured. A significant decline in germination at all concentrations of NaCl indicates that seeds which have been exposed to accelerated ageing are significantly less able to resist even the lowest levels of salt stress. Moreover, the intensity of lipid peroxidation in the seeds and seedlings examined after a year of storage in uncontrolled conditions was higher compared with tests carried out in the year of seed production.

Keywords: lipid peroxidation, oilseed rape, salinity, seed ageing

Experimental and discussion

Seed germination is one of the most critical phases for crop establishment, especially under adverse conditions such as salt stress (Nonogaki *et al*., 2007). There are various endogenous (plant) and exogenous (external environment) factors affecting seed germination under saline conditions, but the most important thing to consider is ageing. Seed ageing or seed deterioration is usually described as a loss of seed quality or viability over time due to adverse environmental factors (Walters *et al*., 2010). Seed vigour is an agronomic trait that describes the seed lot potential for rapid uniform emergence and development under a broad range of field conditions (Ventura *et al*., 2012).

Lipid-rich seeds are generally considered to have limited longevity (Shaban, 2013). Seed storage reserves play an important role in germination and seedling development. Lipid peroxidation (LP) occurs in all the cell membranes when the concentration of reactive oxygen species is high and directly affects normal cell functioning (Kumar *et al*., 2015). The aim of this study was to investigate the effect of different NaCl concentrations on germination after exposing seeds to accelerated ageing conditions, while simultaneously examining the reliability of the accelerated ageing test for assessing seed storage potential.

Four replicates of 100 seeds each of four oilseed rape cultivars (‘Zorica’, ‘Slavica’, ‘Kata’ and ‘Zlatna’), produced at the Institute of Field and Vegetable Crops, Novi Sad, Serbia, were germinated at alternating 20/30°C for seven days (standard germination test (SG)); ISTA, 2015. Filter papers were used as growing media and were regularly moistened with water or 100, 150 or 200 mmol l^{-1} NaCl. At the end of that period, germination percentage and seed malondialdehyde (MDA) content were determined. All tests were conducted after seed harvest and repeated after a year of seed storage under uncontrolled conditions. The accelerated ageing test (AA test) was performed according to the procedure described by Hampton and TeKrony (1995) with minor modification as described by Jovicic *et al*. (2014). The moisture of seed samples were determined according to ISTA (2015) and ranged from 10 to 11%. The seeds were placed in metal boxes and then in water bath at 39°C and 100% RH for 72 hours after which they were subjected to SG tests, as described previously. The degree of LP was determined separately for seeds (after 24 hours exposure to stressful conditions of water bath), seedling shoots and seedling roots. LP was estimated by thiobarbituric acid, measuring malondialdehyde production using a spectrophotometric assay (Ng *et al*., 2000).

Different NaCl concentrations significantly affected the germination of oilseed rape seeds in the SG test, as well as in the AA test, at both times of testing (figure 1). The cultivar ‘Zorica’ in control and all NaCl concentrations showed the highest values in both tests, while cultivar ‘Zlatna’ showed the lowest germination. According to Kaymakanova (2009), salinity affects germination in two ways: 1) a large amount of salt reduces osmotic potential to the point that slows or prevents the water absorption necessary for the mobilization of nutrients required for germination; 2) salts often contain ions (in the case of this experiment, Na^+), which can be toxic to the seed embryo. In addition, Mohammadi (2009) considered that the reduction in seed germination at high NaCl concentrations is primarily caused by disturbances in osmotic regulation leading to difficulties in water adsorption under saline conditions and toxic effects of Na^+ and Cl^- ions. In contrast, Zhang *et al*. (2010) state that the reasons for non-germination of seeds under salt stress conditions are not entirely clear; they suggest that osmotic restriction of water absorption is probably not the main cause of decreased germination. In such circumstances, the integrity of the membrane may be compromised, or the accumulation of salts at the tonoplast can cause loss of function of Na^+/H^+ antiporter, which prevents germination. Although cultivar ‘Zlatna’ showed the lowest germination in the optimal conditions, this cultivar had the smallest germination decline in the AA test for all NaCl treatments, compared with the same NaCl treatments in the SG test, indicating its tolerance to AA. These results confirm the allegations from the literature that the initial germination obtained by the SG test may not be indicative of the seed storage potential.

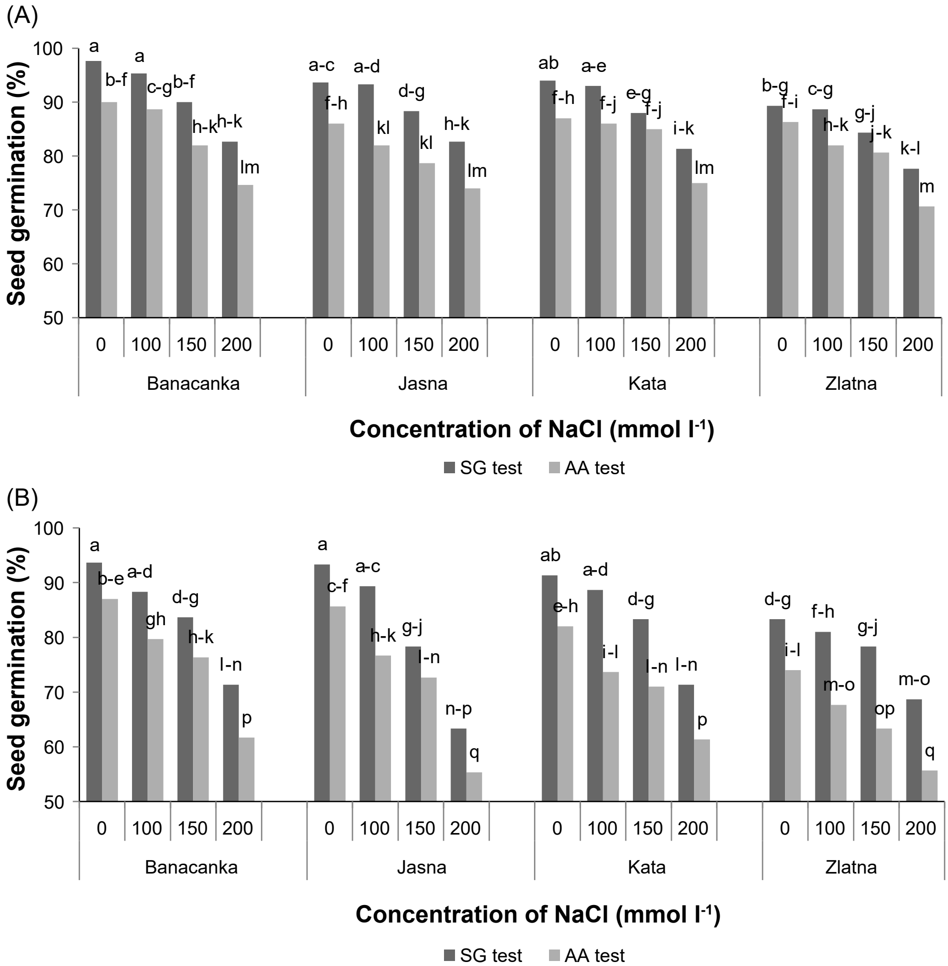

Figure 1. The effect of NaCl on seed germination after harvest (**A**) and after one year of storage (**B**) for seeds of four cultivars of oilseed rape. Values in rows marked with different letters were significantly different according to Duncan *t*-tests $P < 0.05$. SG test = standard germination test; AA test = the accelerated ageing test.

In support of this, the results obtained from the cultivar 'Kata' show that the germination was significantly lower in the AA test than in the SG test: there were no statistically significant differences between controls and NaCl concentrations of 100 and 150 mmol l^{-1}. This cultivar is obviously more sensitive to high temperature and relative humidity than NaCl. These results indicate that there are different responses to various stress conditions between cultivars. There are two main factors that affect seed lot vigour: environmental conditions during production and ageing. For all cultivars, in tests conducted immediately after harvest, significant differences in germination were observed between the control and the lowest concentration of 100 mmol l^{-1} NaCl, but significant decreases occurred only at the higher salt concentrations of 150 and 200 mmol l^{-1}, confirming that oilseed rape is moderately tolerant to salt stress. During the AA test, seeds absorb water from the humid environment and the moisture content of the seeds increases. Combined with a high temperature, this leads to rapid ageing and deterioration (ISTA, 2015). After exposure to seed ageing conditions, the process of germination under salinity was significantly reduced for all the cultivars. The main reason for seed deterioration and the reduction of germination percentage is the influence of high temperature on coagulation and denaturation of proteins (Marcos Filho, 2015). Further, Walters *et al*. (2010) concluded that the level of seed deterioration is determined by seed moisture, storage temperature and seed characteristics that are influenced by genetic and environmental interactions during seed maturation, harvest, and storage. A significant decline in germination at all concentrations of NaCl indicates that seeds that have been exposed to accelerated ageing, are significantly less able to resist even the lowest levels of salt stress. Following one year of storage, there was a reduction in germination of 1-6% (control) and 4-20% (NaCl treatment) in the SG test, and 0-12% (control) and 5-19% (NaCl) in the AA test.

To determine whether different concentrations of NaCl cause oxidation damage in seeds and seedlings of oilseed rape, the LP intensity was measured and expressed as MDA. The amount of MDA, the end product of oxidation of polyunsaturated fatty acids, is used to measure the degree of membrane LP as an indicator of oxidative stress (Yang *et al*., 2012). This can be used as an indicator of plant tolerance and sensitivity to salinity (Jain *et al*., 2001). The results of the experiment showed that the intensity of LP depends on the level of salt stress, in both testing times. LP intensity in seeds after harvest did not show any trend, while after one year of seed storage, LP intensity was salinity-dependent (figure 2). Moreover, the extent of LP in the seeds and seedlings after a year of storage was higher compared with tests carried out in the year of seed production. This shows that the degree of seed damage during storage in uncontrolled conditions was quite high.

Although the seedlings had been exposed to the salt for seven days this was not reflected in a higher LP intensity in the seedlings compared with the seeds. This might suggest that some defense mechanisms were activated in the seedlings that successfully overcame the increased amount of salt ions. Yuan *et al*. (2009) considered that this may be due to the nutrients Na^+ and $C1^-$ or sufficient reserves in the cotyledons that allow the seedlings to adapt to osmotic stress by maintaining turgor.

Lower LP intensity in all cultivars in the first year of the experiment compared with measurements taken after one year of storage in uncontrolled conditions indicates that seeds and seedlings are better protected against oxidative stress caused by saline conditions in the year of seed production. The same conclusion can be drawn analysing the LP intensity in the seeds compared with the seedlings.

In post-storage testing, a strong negative correlation between germination percentage and intensity of LP was observed in both tests, and in both the seeds and the seedling shoots and roots. There was a very significant positive correlation between the results of the AA test conducted after harvest and the germination percentage after one year of storage in uncontrolled conditions ($r = 0.85$; $P < 0.05$) indicates the significance of this test in assessing the storage potential of cultivars and for the prediction of seed behaviour under unfavourable production conditions.

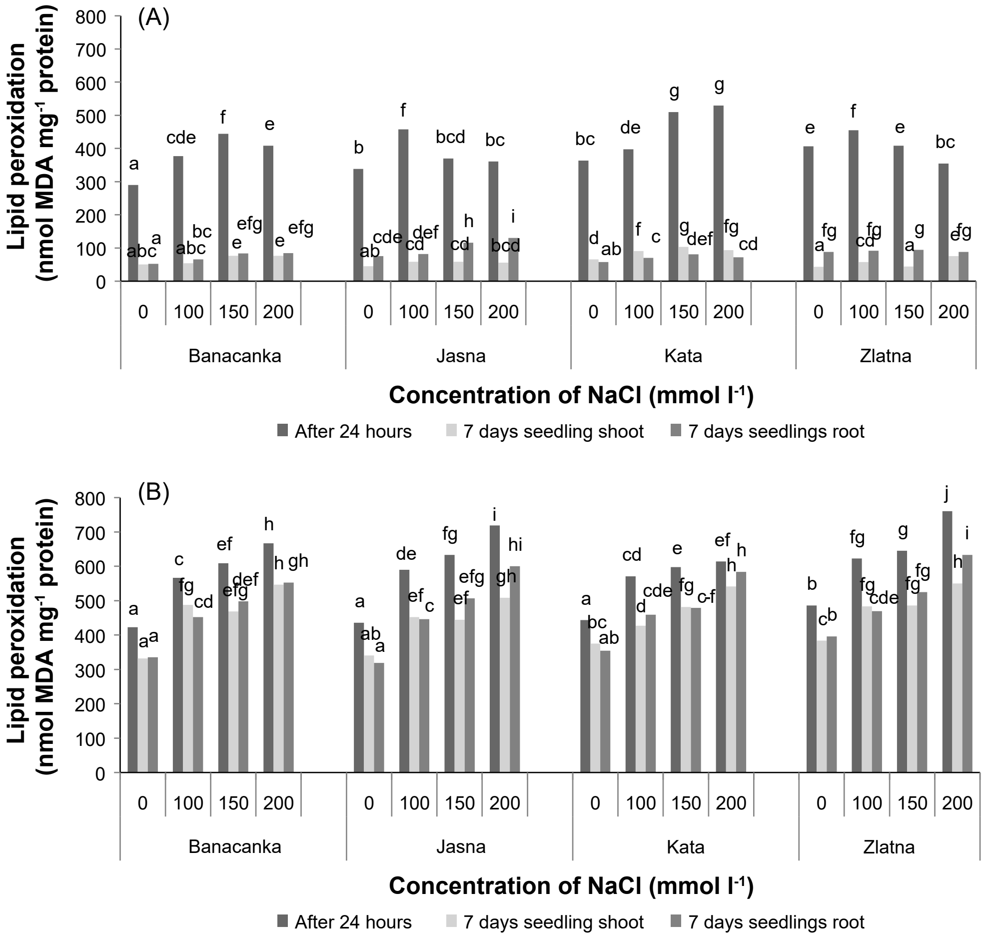

Figure 2. The effect of NaCl on lipid peroxidation (LP) after harvest (**A**) and after one year of seed storage (**B**) for seeds of four varieties of oilseed rape. Values in rows marked with different letters were significantly different according to Duncan *t*-test $P < 0.05$.

The present study clearly showed that the degree of seed deterioration influenced seed response to salinity, and there was substantial genetic variation in salt tolerance among the oilseed rape cultivars. In addition, uncontrolled storage conditions, as well as high temperature and high relative humidity in the AA test, adversely affected germination and LP intensity. The negative correlation between the LP intensity and seed germination indicates that the degree of peroxidation of lipid membranes is of great importance for normal processes in cells under stress conditions during the process of germination.

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