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Salicylic acid seed priming boosts germination in *Brassica rapa* ssp *pekinensis* under cold stress

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Abstract

In Korea, the seeds of *Brassica rapa* ssp. *pekinensis* are sown in the winter and spring months. Cold stress can result in irregular seed germination and poor seedling establishment in some cases. Priming seeds with salicylic acid (SA) can reduce the likelihood of poor seedling establishment when exposed to cold stress conditions. The present study was carried out to determine the effectiveness of seed priming with SA on seed germination and seedling establishment in *B. rapa* ssp. *pekinensis* under cold stress conditions. Seeds were soaked in different SA concentrations (0.5 and 1.0 mM) for 12 and 24 hours, respectively, and then both primed and unprimed seeds germinated at 5 °C. Using SA treatment, seed germination was improved, as was root and shoot length, which were both longer in SA primed seeds than in unprimed seeds. Based on the findings, it is possible to use SA priming as one of the most effective ways to maintain productivity in *B. rapa* ssp. *pekinensis* while it is exposed to low temperatures.

Key words: Chilling stress, Chinese cabbage, plant growth regulator, seed treatment

Introduction

Brassica rapa ssp. *pekinensis*, Chinese cabbage, is used as the main ingredient of Kimchi enjoyed by most Koreans. In Korea, *B. rapa* accounts for 37 % of the leafy and root vegetables, and it is undoubtedly regarded as the most important vegetable in Korea. Fresh *B. rapa* is available all the year round in Korea, indicating that it is also grown in the late winter and early spring. Although *B. rapa* grows in warm regions of Korea during winter and spring, it is frequently exposed to cold temperatures, affecting seed germination negatively. Poor seed germination causes a significant reduction of production (Lamichhane *et al.*, 2018), thus it is essential to make strategies that improve the establishment and growth of seedlings against cold stress to ensure the successful production of *B. rapa*.

Seed priming is a controlled hydration procedure that leads to a set of metabolic changes during the early phases of germination before protrusion of the radicle (Raj and Raj, 2019). These changes enable the seed, rapid and uniform seedling establishment after planting when exposed to abiotic stresses (Feghhenabi *et al.*, 2020; Marthandan *et al.*, 2020). It has been reported that seed priming can also be an effective way to promote seedling emergence and growth under low-temperature conditions (Cao *et al.*, 2019; Fu *et al.*, 2020; Aziz and Peksen, 2020). Among various treatment methods, seed priming with salicylic acid (SA) has been successfully applied in different crop species (Ansari and Zadeh, 2012; Khandan-Mirkohi *et al.*, 2017; Mahmood-ur-Rehman *et al.*, 2020).

However, there is no report on the usefulness of seed priming with SA in *B. rapa* seeds under cold stress conditions. Several studies have shown that the efficiency of SA priming varies among crop species or under different stresses (Khan *et al.*, 2019; Ghafoor *et al.*, 2020; Kulak *et al.*, 2021). It has been suggested that genotype, concentration and duration of seed priming are main factors to decide it (Singh *et al.*, 2018; Azary *et al.*, 2020). We assumed that SA priming could be useful for seeds of *B. rapa*, but the effect of SA priming on the improvement of seed germination and seedling establishment could be different from the conditions of seed priming. Hence, the purposes of the present study were to examine the possibility of overcoming cold stress by seed priming with SA and investigate factors responsible for the improvement of germination and seedling establishment of *B. rapa* under cold stress conditions.

Materials and methods

Germination characteristics: This study was conducted in the Plant Breeding Laboratory, Gangneung-Wonju National University, Korea. Seeds of *B. rapa cv.* Chun-Kwang and *cv.* Jang-Chun were purchased from Sakata Korea, Seoul, Korea. For this experiment, they were subjected in solution with different SA concentration (0.5 and 1 mM) and treated with different application duration (12 and 24 hours). After priming treatment, seeds were rinsed four times with distilled water, and they were air dried for 24 hours at 25 °C.

Germination experiment was conducted in an incubator at 5 °C in 9 cm Petri dishes. 30 seeds from each treatment were placed on double-layer filter paper containing 5 mL of distilled water, and germinated in the incubator. This experiment was laid out in a complete randomized design with 4 replicates, and all treatments were arranged to the experimental unit at random.

Number of germinated seeds was recorded at 24-hour intervals for 10 days, and seed was considered germinated when the radical pierced the coats up to 1 mm. Additional data representing seed germination behavior, such as the final germination percentage

Table 1. Effect of seed priming on seed germination in *B. rapa* under cold stress conditions

Priming condition	Final germination percent			Days to start germination			Mean germination time			Time to 50 % germination		
	Chun-Kwang	Jang-Chun	Mean	Chun-Kwang	Jang-Chun	Mean	Chun-Kwang	Jang-Chun	Mean	Chun-Kwang	Jang-Chun	Mean
Control	8.0 c[z]	38.0 c	23.0 b	8.2 a	6.8 a	7.5 a	8.5 a	8.3 a	8.4 a	8.3 a	7.7 a	8.0 a
0.5 uM 12h	33.0 ab	61.0 a	47.0 a	5.0 c	5.3 b	5.2 b	7.5 b	7.7 a	7.6 b	7.2 b	7.3 a	7.2 b
0.5 uM 24h	19.0 bc	64.0 a	41.5 ab	6.3 bc	5.0 b	5.7 b	8.0 ab	7.7 a	7.9 ab	7.6 ab	7.1 a	7.4 ab
1.0 uM 12h	35.0 a	51.0 ab	43.0 ab	5.5 bc	5.8 b	5.7 b	7.8 ab	8.2 a	8.0 ab	7.3 ab	7.7 a	7.5 ab
1.0 uM 24h	34.0 ab	46.0 ab	40.0 ab	6.5 b	5.3 b	5.9 b	8.3 ab	7.7 a	8.0 ab	7.7 ab	7.2 a	7.4 ab
Mean	25.8	52.0	38.9	6.3	5.6	6.0	8.0	7.9	8.0	7.6	7.4	7.5

[z] Different letters within columns indicate the mean separation by Duncan's multiple range test *(P* = 0.05).

(FGP), days to start germination (DSG), mean germination time (MGT) and the time to 50% germination (T_{50}), was also analysed.

FGP was calculated following the formula.

FGP = (Number of seeds germinated on final day × 100)/ Total number of seeds sown

MGT was measured according to the equation of Ellis and Roberts (1981) described as below.

$MGT = \Sigma Dn / \Sigma n$

In this equation, 'n' represents the number of germinated seeds per day and D indicates the number of days counted from the beginning of emergence. T_{50} was calculated following the formulae of Coolbear *et al.* (1984).

Seedling establishment: The effect of priming with SA on seedling vigour was evaluated at the end of seed germination experiment. Length of seedlings was measured with an electronic caliper 10 days after sowing. Fresh and dry weight of germinated seedlings were also investigated. For dry weight measurement, the seedlings were dried in an oven at 80 °C for 72 h, and then dry weights were obtained (Jang *et al.*, 2020). Afterward, the seedling vigor index was estimated according to equation of Orchard (1977).

SVI = [seedling length (cm) × germination percentage]

Statistical analysis: The data were analyzed by SPSS software 24.0 (SPSS Inc, Chicago, IL) and means were compared by Duncan Multiple Range Test.

Results and discussion

Germination characteristics: Under cold stress conditions, FGP ranged from 7.2 to 64.0 %, and it depended on cultivars or whether priming was performed or not (Table 1). In the control group, the FGP of *B. rapa cv.* Jang-Chun (38.0 %) was much higher than that of *B. rapa cv.* Chun-Kwang (7.2 %). It has been frequently reported that cold tolerance in plants differs among genotypes within the same species (Arisz *et al.*, 2018; Ghimire *et al.*, 2019; Rativa *et al.*, 2020). The first stage of plant establishment is seed germination, and germination in less abiotic tolerant genotypes is more easily fail under unfavorable growth conditions. Hence, it was assumed that this result might confer different cold tolerance between two cultivars. The highest FGP was found in the seeds primed with 0.5 uM of SA in *B. rapa cv.* Jang-Chun while the highest FGP was investigated in the seeds primed with 1.0 uM of SA in *B. rapa cv.* Chun-Kwang. These results indicate that SA priming commonly improves the FGP, but the effect of SA priming on the FGP differs between two cultivars (Table 1). For example, FGP at 0.5 uM of SA for 12 hours was 33 % and at 1.0 uM concentration of SA it was 34 % after 24 hours of SA treatment in *B. rapa cv.* Chun-Kwang. Thus, it seemed that FGP in *B. rapa cv.* Chun-Kwang was not quite differently affected by concentration and duration of seeds to SA priming. By contrast, FGP in *B. rapa cv.* Jang-Chun decreased by increasing the priming concentration and duration, showing that optimal priming condition for improvement of FGP could be different depending on genotypes in *B. rapa.*

Pre-application of SA at 0.5 uM shortened MGT and T_{50} compared to control regardless of cultivars. Farroq *et al.* (2008) and Mahmood-ur-Rehman *et al.* (2020) also reported that MGT

Table 2. Effect of seed priming on seedling establishment in *B. rapa* under cold stress conditions

Priming condition	Length of seedling			Fresh weight			Dry weight			Seedling vigor index		
	Chun-Kwang	Jang-Chun	Mean	Chun-Kwang	Jang-Chun	Mean	Chun-Kwang	Jang-Chun	Mean	Chun-Kwang	Jang-Chun	Mean
Control	0.43 c[z]	0.52 b	0.47 b	41.03 c	104.62 a	72.83 b	11.90 c	31.15 c	21.53 b	3.4 c	19.4 c	11.4 ab
0.5 uM 12h	0.55 ab	0.77 a	0.66 a	124.53 a	169.90 a	147.22 a	34.30 a	49.08 a	41.69 a	18.0 ab	47.1 a	32.6 a
0.5 uM 24h	0.50 bc	0.59 ab	0.55 ab	82.43 b	170.05 a	126.24 a	23.57 b	49.08 a	36.33 a	9.5 bc	38.2 ab	23.9 ab
1.0 uM 12h	0.66 a	0.46 b	0.55 ab	122.83 a	136.75 a	129.79 a	33.30 a	41.20 ab	37.23 a	22.6 a	23.0 bc	22.8 ab
1.0 uM 24h	0.55 ab	0.49 b	0.52 b	115.83 a	126.43 a	121.12 a	33.07 a	36.53 ab	34.80 a	18.8 a	23.3 bc	21.0 ab
Mean	0.54	0.57	0.55	97.33	141.55	119.44	27.23	41.41	34.32	14.5	30.2	22.3

[z] Different letters within columns indicate the mean separation by Duncan's multiple range test *(P* = 0.05).

and T_{50} decreased with SA priming in carrot and hybrid maize. SA is involved in essential physiological processes for survival of plants such as photosynthesis and metabolism, and provides the ability to protect plants against diverse abiotic stresses by enhancing antioxidant and water utilization capacity (Ahmad *et al.*, 2019; Mohamed *et al.*, 2020). It has been well proposed that SA priming on the seed germination stage also has a positive effect on plants. SA priming mediates cell division in germinating seeds as it acts as germination inducer, and improves germination behaviour by modifying antioxidant defence system under cold, drought and salt stress (Shatpathy *et al.*, 2018; Shaikh-Abol-Hasani and Roshandel, 2019; Jeammuangpuk *et al.*, 2020). From this experiment, statistically faster DSG were also observed with all SA priming conditions. Hence, early and better germination observed in seeds primed with SA strongly suggests that SA contributes to better metabolic preparation for seed germination and to induction of antioxidant responses for protection from dehydration damage during seed germination in *B. rapa* under cold stress conditions.

Seedling establishment: In un-primed seeds, *B. rapa cv.* Jang-Chun had higher seedling length and weight compared with *B. rapa cv.* Chun-Kwang under cold stress condition (Table 2). All SA priming conditions significantly resulted in the increase of shoot length in the case of *B. rapa cv.* Chun-Kwang, whereas only specific priming conditions had a positive impact on *B. rapa cv.* Jang-Chun. All SA priming conditions, however, increased the fresh and dry weight of seedlings in both cultivars. In overall, SA priming seemed to have a positive impact on initial seedling development under cold stress conditions.

Although the highest value in fresh and dry weight was commonly observed from *B. rapa cv.* Jang-Chun, SA priming had a huge impact on *B. rapa cv.* Chun-Kwang in the increase of fresh and dry weight when compared to control. For instance, fresh and dry weight in seeds primed with 0.5 uM of SA for 12 hours increased by approximately 1.7 times in *B. rapa cv.* Jang-Chun, but fresh and dry weight in seeds primed with the same condition increased by 3 times in *B. rapa cv.* Chun-Kwang compared with un-primed seeds. It was presumed that different cold tolerance between two cultivars resulted in different responses to SA during seedling growth, and SA priming could be very helpful to seedling establishment for less cold tolerant plants.

Like other results obtained from this study, higher SVI value was observed in *B. rapa cv.* Jang-Chun compared to *B. rapa cv.* Chun-Kwang. In *B. rapa cv.* Jang-Chun, seed priming at 0.5 uM of SA for 12 hours was more effective than other priming conditions while pre-application at 1.0 uM of SA for 12 hours was the most effective condition in *B. rapa cv.* Chun-Kwang, showing that the effect of SA priming on SVI differed from cultivars (Table 2). Apart from this result, SA priming significantly increased the SVI in both cultivars. The present result was in conformity with the results of many other researchers. In rice, maize and periwinkle, seeds primed with SA had better germination pattern and showed a higher level of seedling vigour than non-primed seeds under cold stress conditions (Imran *et al.*, 2013; Pouramir-Dashtmian *et al.*, 2014; Khandan-Mirkohi *et al.*, 2017). It has been reported that SA priming induces the promotion of seedling growth by increasing the level of cell division within the apical meristem of seedling root and by allowing protection from the damage of cell membrane caused by cold stress (Farooq *et al.*, 2008; Gharib and Hegazi, 2010). Hence, results from this investigation suggest that SA also has a growth promoting and protective effect on seedlings of *B. rapa* under cold stress conditions.

The present study clearly demonstrates that SA priming is statisically effective at inducing cold stress tolerance at the stage of seed germination and seedling establishment in *B. rapa*. Interestingly, SA priming conditions and cultivars significantly affected the days to start germination and the seedling vigor index of *B. rapa* at a 1 % probability level while no significant variation was found in the interaction of cultivars and priming conditions on the final germination percent, fresh weight and dry weight. We observed that there were quite big differences in mean data obtained from two cultivars, although it could be confirmed that SA priming was clearly effective when we analyzed them separately by cultivar. These results suggest that SA priming has a positive effect on the improvement of seedling establishment behaviour for *B. rapa* under cold stress conditions, but the effect of SA priming treatment may vary greatly depending on the cultivars. Other researchers also found that the response of seed priming was cultivar-dependent (Goro and Sinha, 2020; Khaing *et al.*, 2020).

In conclusion, it should be noted that the response of SA priming depends on cultivars within *B. rapa*, and finding out the optimum SA priming procedure for the specific cultivar is also vital before the sizeable commercial application.

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