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Priming with smoke-derived karrikinolide enhances germination and transplant quality of immature and mature pepper seed lots

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ABSTRACT

The aim of this study was to test the effect of the smoke-derived compound, karrikinolide (KAR_1), and hydropriming treatments on the seed germination of pepper (*Capsicum annuum* L.). The effect of priming treatments on seedling emergence percentages and rates, seedling fresh and dry mass, catalase (CAT), superoxide dismutase (SOD) and ascorbate peroxidase (APX) activity of immature and mature seed lots for four pepper cultivars (Kandil D., Surmeli, Yaglık and Corbacı) harvested in 2015 and 2016 were evaluated. Priming with 10^{-7} M KAR_1 enhanced seed germination and seedling emergence for seeds from both harvest periods, and was more influential on immature seeds than mature seeds. Specifically, priming with KAR_1 improved germination by more than 20% in immature seeds, compared to around 10% in the mature seeds. The differences in seedling emergence were 18% and 22% in 2015, and 21% and 22% in 2016, respectively. Priming with KAR_1 increased catalase activity, but reduced APX and SOD activity in both seed lots for cultivar Corbacı. Thus, results indicate that priming with KAR_1 can be used as an enhancing treatment in the cultivation of pepper seeds, and that the benefit is greater in immature seeds than in mature seeds, regardless of the cultivar or year of harvest.

1. Introduction

Pepper (*Capsicum annuum* L.) is a warm climate vegetable crop, the seeds of which are sown in modules for transplant production in early spring in Mediterranean regions. Slow or sporadic germination and seedling emergence are common phenomena in transplanting modules resulting in weak and non-uniform sized plants (Demir et al., 2008). Rapid germination and emergence in modules requires high-quality seeds which are influenced by pre-and post-harvest production factors (Taylor et al., 1998). Pepper is a subtropical crop with a continuously flowering structure. The pepper fruits form at different levels of the plant and seed maturation occurs acropetally (Demir and Ellis, 1992). The maturation stages during the seed development period have an influence on seed germination and emergence potential in pepper (Demir and Ellis, 1992). In commercial seed production activities, the pepper fruits for seed production are harvested from the plant at the same time (once-over mechanical harvesting) and seeds are extracted. Such once-over mechanical harvesting of continuously flowering vegetable species, including pepper, is one pre-harvest factor determining the overall performance of the seed lot. Commercial seed lots from once-over mechanically harvested crops comprise seeds with a mixture of the degree of maturity extracted from fruits at the same time. Seeds that are immature from the same lot have a lower performance (i.e. slow emergence, weak seedlings) when compared with mature, fully ripe seeds in germination and transplant production (Demir et al., 2008).

The priming of seeds with osmotica (mannitol, polyethylene glycol), inorganic salts (sodium chloride, nitrate and phosphate salts of potassium) or water enhances seedling quality in a variety of crops, including pepper (Khan, 1992). Priming involves the hydration of seeds in the above solutions, which starts the preliminary process of germination (Khan, 1992) and is a commercially accepted method for improving and synchronizing germination, and enhancing seedling vigor.

Smoke or aqueous smoke extracts may also potentially be used as a priming agent to enhance seed germination and seedling vigor in many plants (Brown and Van Staden, 1997; Light and Van Staden, 2004; Van Staden et al., 2006). The chemical identity of one of the known active compounds present in smoke is 3-methyl-2*H*-furo[2,3-*c*]pyran-2-one, a butenolide-type compound referred to as karrikinolide (KAR_1) (Flematti et al., 2004; Van Staden et al., 2004; Chiwocha et al., 2009). The promotive role of KAR_1 on seed germination and seedling vigor has been documented (Van Staden et al., 2006), with KAR_1 having been previously shown to increase transplant quality in commercial pepper seed lots (Demir et al., 2012). The physiological basis of KAR_1-priming may relate to an increase in replicated DNA (Jain and Van

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Table 1
Germination percentages for four cultivars of immature or mature pepper seeds, primed with karrikinolide (KAR_1, 10^{-7} M) or hydropriming treatments. Means with different letters in the same line (i.e. same year, maturity stage and cultivar) are significantly different at 5% level with Duncan's multiple range test. Values are means ± S.D. (n = 4).

Year	Cultivar	Germination (%)					
		Immature			Mature		
		KAR_1	HP	C	KAR_1	HP	C
2015	Kandil D.	69 ± 0.67 a	52 ± 1.15 b	41 ± 0.67 c	75 ± 0.67 a	68 ± 2.00 b	59 ± 1.76 c
	Surmeli	50 ± 2.00 a	43 ± 2.67 ab	37 ± 2.67 b	86 ± 1.15 a	85 ± 0.67 a	80 ± 1.15 b
	Yaglık	81 ± 0.67 a	73 ± 0.67 a	55 ± 1.33 b	82 ± 1.15 a	74 ± 1.15 b	71 ± 1.33 b
	Corbacı	91 ± 1.76 a	81 ± 0.67 b	76 ± 1.15 c	87 ± 0.67 a	85 ± 0.67 ab	83 ± 0.67 b
2016	Kandil D.	65 ± 0.67 a	50 ± 0.00 b	33 ± 0.67 c	70 ± 0.67 a	64 ± 1.15 a	55 ± 1.33 b
	Surmeli	48 ± 0.00 a	35 ± 1.76 b	25 ± 1.76 c	83 ± 0.67 a	77 ± 1.76 b	74 ± 1.15 b
	Yaglık	77 ± 0.67 a	70 ± 1.15 b	50 ± 1.15 c	81 ± 0.67 a	75 ± 0.67 b	70 ± 1.15 c
	Corbacı	81 ± 0.67 a	68 ± 1.15 b	67 ± 1.76 b	87 ± 0.67 a	85 ± 1.33 b	83 ± 0.67 c

KAR_1: karrikinolide; HP: hydroprimed; C: control.

Staden, 2007), induction of the cell division cycle (Soos et al., 2009), or action similar to gibberellins (Long et al., 2010). KAR_1-priming is also associated with an enzymatic activity, i.e. catalase (Demir et al., 2012) or superoxide dismutase, through the degradation of storage compounds, since these enzymes can protect against damage to the cell processes during priming caused by reactive oxygen species (Bailly et al., 2001).

In previous smoke priming studies, mature seeds are typically used. However, any effect of smoke or KAR_1 on less mature seeds is not well known. As described above, in commercial seed production companies, seeds at different stages of maturity may be mixed in the same seed lot/batch. In this regard, if the performance of the less developed seeds is improved, then it may be reflected in the overall performance of the seed lot. The present study was undertaken to assess the potential of smoke-derived KAR_1 as a priming agent for immature and mature pepper seeds of four cultivars, with the aim being to investigate the increase in the overall germination and seedling emergence percentages of the seed lots tested.

2. Materials and methods

Pepper seeds (*Capsicum annuum* L.) of Kandil D., Surmeli, Yaglık and Corbacı cultivars were grown between May and September in 2015 and 2016 at the experimental field of the Central Horticultural Research Institute/Ministry of Food, Agriculture and Livestock/Yalova/Turkey. The soil is a sandy loam, and plant spacing was 100 cm between plants and 60 cm between rows. Before planting, 10:10:10 NPK at a rate of 60 kg per 1000 m^2 was applied to the rows, and an additional 15 kg of ammonium sulfate and 15 kg of potassium nitrate were applied one month after transplanting for the same area. The field was irrigated once a week until July, and then every three days until the end of the experiment. A total of 100 plants were tagged at full anthesis and fruits were harvested 50–55 (immature), 65–70 days (mature) after anthesis for each cultivar in both years. Seeds were extracted from the fruits and dried at 25 °C in an incubator for 24 h to about 8% seed moisture content. The dried seeds were stored in the dark at 5 °C in hermetically laminated aluminum foil packets until used.

The experiments were carried out between September and December 2016. The KAR_1 used in the experiment was isolated (99% purity) from smoke-saturated water, as described by Van Staden et al. (2004). For the priming treatments, 800 pepper seeds from each seed lot were placed in 90-mm Petri dishes (200 seeds per Petri dish) on two layers of filter paper (Whatman No. 5), moistened with 6 mL of test solution. The test solutions were either distilled water (hydropriming) or 10^{-7} M KAR_1. This concentration was previously shown to be suitable as a test concentration (Kulkarni et al., 2006; Jain and Van Staden, 2007). Petri dishes were wrapped in cling film and aluminum foil and kept in the dark at 25 °C for 40 h. The Petri dishes were not opened throughout the priming treatment, and the incubator door was kept closed. Following priming, the seeds were rinsed under tap water for 30 s and dried at room temperature for 24 h. All processing was conducted under very dim light (about 1 μmol m^{-2} s^{-1}). The germination tests of the primed (hydro-and KAR_1-priming) and control (non-primed) seeds (four replicates of 50 seeds from each seed lot for each treatment) were assessed using the 'between paper' method (ISTA, 2016) at 25 °C in the dark. The percentage of normal seedlings was determined after 14 days.

For the seedling emergence tests, four replicates of 50 seeds from each priming treatment and maturation stage (i.e. 4 cultivars × 3 treatments × 2 harvests) were sown at a depth of 2 cm in modules containing a peat moss medium (Plantaflor, Germany). The modules were placed in a climate-controlled room at 23 ± 2 °C and illuminated with 170 μmol m^{-2} s^{-1} light provided by white fluorescent lamps. The room was kept at about 70% RH to ensure minimum evaporation. Emergence counts were made every day for 20 days. At the end of the experiment, seedling emergence (the appearance of hypocotyls at the surface) was monitored by counting twice daily (for the first 10 days), then once a day thereafter. After 20 days, the seedling emergence percentages (the appearance of hypocotyls at the surface) were recorded

Table 2
Seedling emergence of normal seedlings for four cultivars of immature or mature pepper seeds, primed with karrikinolide (KAR_1, 10^{-7} M) or hydropriming treatments. Means with different letters in the same line (i.e. same year, maturity stage and cultivar) are significantly different at 5% level with Duncan's multiple range test. Values are means ± S.D. (n = 4).

Year	Cultivar	Seedling emergence (%)					
		Immature			Mature		
		KAR_1	HP	C	KAR_1	HP	C
2015	Kandil D.	81 ± 0.67 a	68 ± 2.31 b	60 ± 1.15 c	86 ± 2.00 a	83 ± 2.40 a	54 ± 2.31 b
	Surmeli	74 ± 1.15 a	63 ± 0.67 b	53 ± 1.76 c	83 ± 0.67 a	81 ± 0.67 a	73 ± 0.67 b
	Yaglık	88 ± 1.15 a	79 ± 3.71 ab	73 ± 4.81 b	91 ± 4.16 a	86 ± 0.00 a	67 ± 1.33 b
	Corbacı	93 ± 1.33 a	83 ± 1.76 b	80 ± 1.15 b	89 ± 0.67 a	84 ± 0.00 b	68 ± 1.15 c
2016	Kandil D.	85 ± 0.67 a	72 ± 0.00 b	63 ± 1.76 c	87 ± 0.67 a	81 ± 0.67 a	53 ± 1.76 b
	Surmeli	77 ± 1.33 a	65 ± 0.67 b	53 ± 3.33 c	85 ± 1.76 a	78 ± 3.06 a	70 ± 1.15 b
	Yaglık	81 ± 0.67 a	74 ± 1.15 b	69 ± 2.40 b	89 ± 2.40 a	87 ± 1.33 a	73 ± 2.40 b
	Corbacı	95 ± 1.33 a	83 ± 2.40 b	68 ± 0.00 c	95 ± 1.76 a	92 ± 1.15 a	74 ± 2.31 b

KAR_1: karrikinolide; HP: hydroprimed; C: control.

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