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Positive effects of karrikin on seed germination of three medicinal herbs under drought stress

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ABSTRACT- Improper seed germination is a serious challenge for medicinal herbs under drought stress conditions in semi-arid and arid regions. A new group of plant growth regulators known as karrikins have been proved useful to improve seed germination in some plants. In this regard, the effects of karrikin on seed germination and vigor of three medicinals under drought stress were examined. Seeds of *Trachyspermum copticum* (L.) Link, *Foeniculum vulgare* Miller, and *Cuminum cyminum* L. were sowed under drought treatments (-1, -1.5, -2 and -2.5 MPa of PEG 6000), with and without synthetic Karrikin GR24 (10µM). Results indicated increasing osmotic pressure seed germination characteristics were severely reduced; however, karrikin significantly increased the seed germination of the three herbs. The application of karrikin in all drought conditions significantly increased germination percentage, germination rate, germination index, seedling vigor, shoot length and radicle length of all herb seedlings. Ajwain (*T. copticum*) showed a greater drought tolerance compared to the other medicinal species.

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INTRODUCTION

Drought stress is the major limiting factor for plant production in semi-arid and arid regions. Seed germination of plants, as a serious life cycle and a prerequisite of plant production, is severely affected by water deficiency (Li et al., 2011; Rauf et al., 2007; Rivas Arancibia et al., 2006; Zeng et al., 2010). Subsequently, poor seed germination **causes** weak seedling growth and abnormality and finally reduces plant yield (Mos et al., 2007; Sun et al., 2010; Zeng et al., 2010). Different studies have shown that water stress increases the mean time of seed germination and delays plant production (Kaya et al., 2006; Li et al., 2011). Exogenous use of chemicals that makes the plant tolerant and starts defense responses has been extensively studied (Kissoudis et al., 2014). One of the priming techniques is smoke extract which has been reported to improve seed germination in various plants (Chiwocha et al., 2009; Ghebrehiwot et al., 2013; Coons et al., 2014). This stimulatory effect of smoke on germination has been attributed to karrikinolide activity. Karrikinolide is an active chemical compound of butenolide or 3-methyl-2H-furo [2, 3-c] pyran-2-one formula, which is totally called karrikins, a Noongar word for ‘smoke’ (Chiwocha et al., 2009; Halford, 2010; Long et al., 2011; Nair et al., 2012). Karrikins are a newly discovered group of plant growth regulators (Yamada et al., 2014), effective for removing seed eco-dormancy and improving the germination of many plant species (Halford, 2010; Ghebrehiwot et al., 2013). The beneficial effect of karrikin on seed germination and seedling development is described greater than the effect of fire in the ecology (Chiwocha et al., 2009).

Three important medicinal plants of Iran with magnificent therapeutic properties are *Trachyspermum copticum* (L.) Link (Hedge and Lamond, 1987), *Foeniculum vulgare* Miller (Bahmani et al., 2013) and *Cuminum cyminum* L. (Niazi and Raja, 1971). On the other hand, these herbs risk reduced seed germination due to prolonged drought periods of the semi-arid and arid regions of Iran (Akhavan Armaki et al., 2013). The main objective of this study is to examine karrikin’s role on seed germination of ajwain, fennel and cumin under different drought stress treatments. Furthermore, the most responsive herb to exogenous application of this new PGR is evaluated.

MATERIALS AND METHODS

Experimental Plants and Laboratory Condition

The seeds of *Trachyspermum copticum* (L.) Link, *Foeniculum vulgare* Miller, and *Cuminum cyminum* L. were put in scientific™ sterilin™ standard 90mm Petri dishes in August 2012 with a day/night temperature of 25/20 °C and 55 - 65% relative humidity.

Drought Stress Treatments

Drought stress was implemented using PEG 6000 with osmotic pressures of 0, -1, -1.5, -2 and -2.5 MPa.

Karrikin Treatments

30 ml of freshly prepared synthetic karrikin GR24 solution (Chiralix, The Netherlands) with a stimulating concentration of 10 µM was applied in each Petri dish for seed imbibitions. Each drought stress treatment was accompanied by a control without karrikin.

Measured Characteristics

Germination was determined by 2 mm exposure of radicle, and recorded twice daily for the subsequent 14 days. The germination characteristics shoot and radicle length (Guterres et al., 2013), germination percentage (ISTA, 2008), germination rate (Maguire, 1962), germination index (Tekrony et al., 1991) and seedling vigor (Agrawal, 2002) were measured as follows:
Germination percentage (GP) = S/T × 100
Germination rate (GR) = (Σ Ni/Di)/S
Germination index (GI) = (number of new seedlings after day n1/ n1) + (number of new seedlings after day n2/ n2) +................ + (number of new seedlings after day ni/ ni).
Seedling vigor = (RL+SL) × GP
S: Number of germinated seeds
T: Total number of seeds
Ni: The number of germinated seeds in Di (day)
RL: Root length
SL: Shoot length

Experiment Design and Data Analysis

Experiments were conducted in a complete randomized design with four replicates. Analysis of data was done using SPSS V.19, and means were compared by LSD test of ANOVA at a significant level of P≤ 0.05.

RESULTS AND DISCUSSION

Germination Percentage

Drought stress significantly decreased the germination percentage of the seeds among which the severest decline was observed at -2.5 MPa osmotic pressure. Karrikin application significantly improved the percentage of seed germination of all herbs. A double increase was detected at -1.5 MPa for *T. copticum*. (p < 0.01) (Fig. 1).

Germination Rate

Drought stress significantly decreased the germination rate of the medicinals' seeds among which the severest decline was at -2.5 MPa osmotic pressure. On the other hand, karrikin application significantly improved the rate of seed germination (P < 0.01) (Fig. 2). The extent of the effect of karrikin on the rate and percentage of germination differed between species and drought treatments. The greatest enhancement of germination rate by karrikin was observed at drought level of -2 MPa for *T. copticum*. (Fig. 2).

Germination Index

The germination index of all investigated species was significantly decreased by drought stress among which the utmost decline was seen at -2.5 MPa osmotic pressure (P<0.01) (Fig. 3). Karrikin use in drought stress conditions improved the germination index of all of the herbs significantly (p<0.01), among which the highest effect was obtained at -1.5 MPa for *T. copticum*. (Fig. 3).

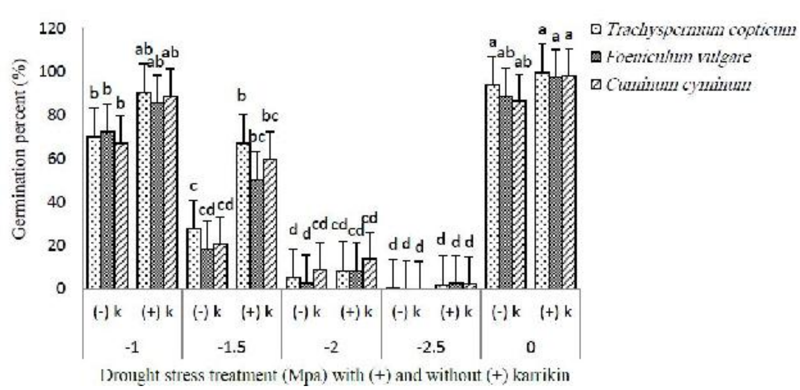

Fig. 1. The effect of exogenous karrikin on germination percentage of herbs' seed under drought stress conditions

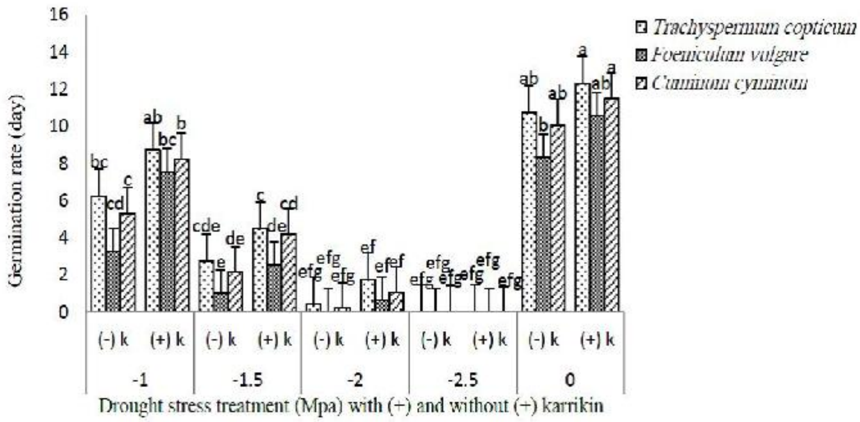

Fig. 2. The effect of exogenous karrikin on germination rate of herbs' seed under drought stress conditions

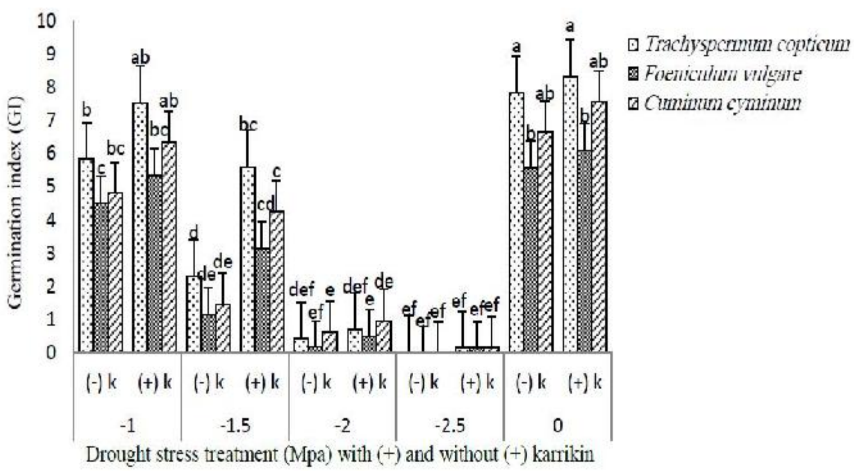

Fig. 3. The effect of exogenous karrikin on germination index of herbs' seed under drought stress conditions

Seedling Vigor

The seed vigor of seeds was significantly deteriorated starting from the lowest level of drought stress. The harsh osmotic conditions greatly reduced the seed vigor of *F. vulgare* compared to the other species. Seed vigor of all species was reduced to near zero at drought level of -1.5 MPa, but the addition of karrikin doubled the seed vigor of the herbs among which the most increase was achieved for *T. copticum*. ($P< 0.01$) (Fig. 4).

Shoot Length

The shoot length of all seeds was significantly diminished by drought stress. The greatest reduction of shoot length was recorded at drought level of -2.5 MPa. The application of karrikin enhanced the shoot length of the seeds among which *F. vulgare* showed the most improvement compared to other herbs. ($p < 0.01$) (Fig. 5).

Radicle Length

The radicle length of all seeds was significantly lessened by all drought stress levels. The greatest decline of radicle length was determined at drought level of -2.5 MPa. The application of karrikin enhanced the radicle length of the seeds among which *T. copticum* showed the most improved growth compared to other herbs ($P < 0.01$) (Fig. 6).

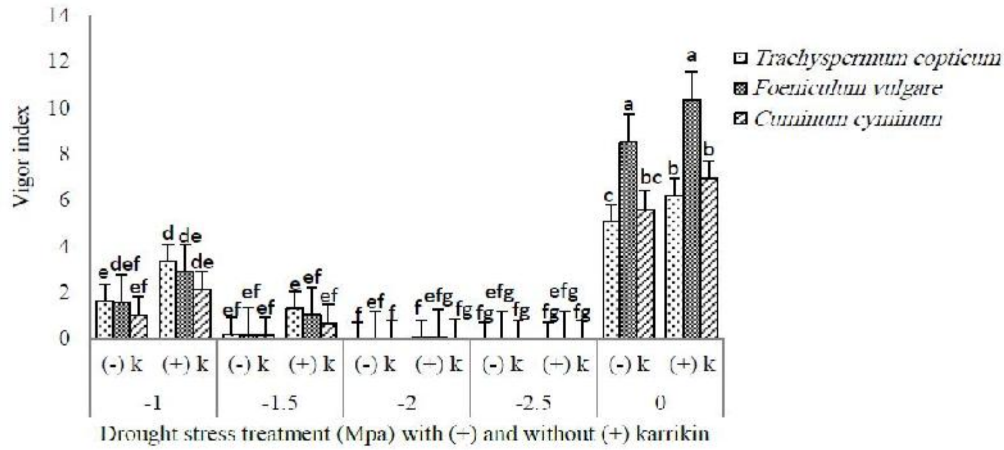

Fig. 4. The effect of exogenous karrikin on seedling vigor of herbs' seed under drought stress conditions

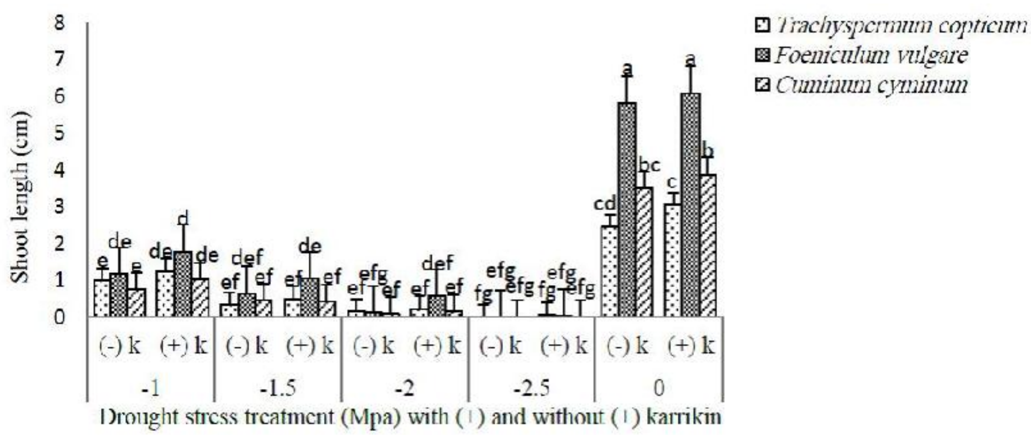

Fig. 5. The effect of exogenous karrikin on shoot length of herbs' seed under drought stress conditions

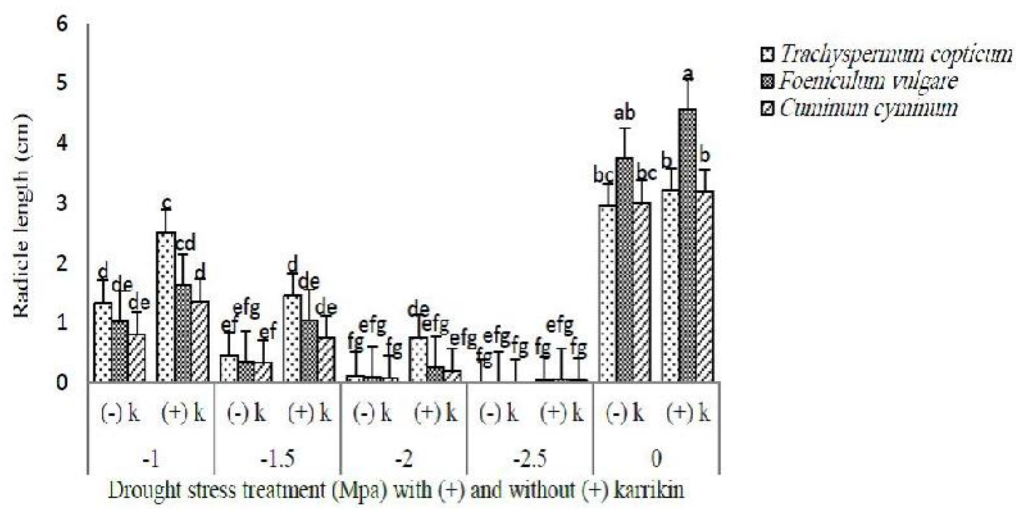

Fig. 6. The effect of exogenous karrikin on radicle length of herbs' seed under drought stress conditions

Drought stress effectively reduced the seed germination and vigor of the medicinal herbs as seen in other plants (Kaya et al., 2006; Van den Berg and Zeng, 2006; Mos et al., 2007; Sun et al., 2010). Various chemicals have been proposed to improve the plants tolerance to drought stress conditions including tryptophan (Rao et al., 2012), glycinebetaine (Giri, 2011; Zhang et al., 2013), humic acid (Zhang et al., 2013), nitric oxide (Rahimian Boogar et al., 2014), brassinosteroid (Behnamnia et al., 2009; Gomes et al., 2013), β-ABA (Jakab et al., 2005; Ton et al., 2005; Li et al., 2011; Hussain et al., 2012), salicylic acid (Habibi, 2012; Rao et al., 2012; Kang et al., 2013; Nazarli et al., 2014) and jasmonates (Alam et al., 2014; Nazarli et al., 2014). Karrikins as new plant growth regulators have previously been shown to facilitate seed germination (Long et al., 2011; Nair et al. 2012; Waters et al., 2012; Cheng et al., 2013). In the current research, also, the application of karrikin positively ameliorated seed germination characteristics of all the herbs under drought stress (Fig. 1-6). This mechanism can be clarified by the point that karrikins decrease the ABA:GA ratio, which is physiologically the main stimulus to enhanced seed germination (Cheng et al., 2013). Sunmonu et al. (2016) revealed that karrikins help seed growth of bean and maize by effective mobilization of starch accumulations from cotyledons/endosperms to different seedling tissues. The likely mode of action by which this is facilitated could be by promoting hydrolytic enzyme activities, mostly amylase (Sunmonu et al., 2016). Moreover, it has been shown that karrikins decrease lipid peroxidation and oxidative enzyme activity, thereby motivating seedling growth (Sunmonu et al., 2016). Ha et al. (2014) reported that exogenous strigolactone treatment had a positive regulatory role in *Arabidopsis* and enhanced drought tolerance of the plants. They showed that plants incorporate multiple hormone-response pathways—at least karrikin, abscisic acid, and cytokinin pathways—for adapting to ecological stress. Their studies proved that genetic regulation of karrikin content/response could provide a new way for the development of crops with advanced drought tolerance (Ha et al., 2014).

In the present study, karrikin had its most significant mitigating effect on seed germination of *T. copticum*. at drought stress conditions (Fig. 1-6). Generally, karrikins have been presented as largely efficient stimulants that increase seed germination of more than 1200 species; however, various species are stimulated differently by this plant growth regulator according to their genetic potentials (Dixon et al., 2009). Therefore, it is crucial that each plant's response to this PGR has to be *de novo* examined. Due to the fact that ajwain is moderately drought tolerant (Rohamare et al., 2014) as compared to other herbs, it might have a superior potential to alleviate oxidative damages by water deficit conditions.

CONCLUSIONS

Although drought stress severely deteriorates the seed germination of all herbs, the results of this study showed mitigating effects of karrikin on improving seed germination of herbs under water deficit conditions. Furthermore, it was found that Ajwain (*T. copticum*) has a greater drought tolerance possibly reducing oxidative damages compared to other medicinal species. Nevertheless, the use of karrikin to alleviate other stresses for the herbs' seed germination and also antioxidant response of this PGR merits further investigations.

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تأثیر مثبت کاریکین بر جوانه زنی بذر سه گیاه دارویی تحت تنش خشکی

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قدرت دانهال

Cuminum cyminum

Foeniculum vulgare

Trachyspermum

چکیده- جوانه زنی نامناسب بذر گیاهان دارویی در مناطق نیمه خشک و خشک یک چالش مهم به حساب می آید. مشخص شده است که یک گروه جدید از تنظیم کننده های رشد گیاهی، معروف به کاریکین ها، برای بهبود جوانه زنی بذر برخی گیاهان مفید هستند. در این راستا تأثیر کاریکین بر جوانه زنی و قدرت دانهال سه گیاه دارویی تحت تنش خشکی مورد بررسی قرار گرفت. بذرهای زنیان (*Trachyspermum copticum* (L.) Link)، رازیانه (*Foeniculum vulgare* Miller) و زیره سبز (*Cuminum cyminum* L.) تحت تیمارهای خشکی (۱-، ۱/۵-، ۲- و ۲/۵ - MPa از PEG 6000) با یا بدون استفاده از کاریکین سنتزی (GR24 (10µM کشت شدند. اگرچه با افزایش فشار اسمزی مشخصات جوانه زنی بذر به شدت کاهش یافت، اما کاریکین بطور معنی داری فاکتورهای جوانه زنی سه گیاه دارویی را افزایش داد. کاربرد کاریکین در تمام شرایط خشکی بطور معنی داری درصد جوانه زنی، سرعت جوانه زنی، شاخص جوانه زنی، قدرت دانهال، طول شاخساره و طول ریشه چه تمام دانهال های گیاهان دارویی را افزایش داد. زنیان (*T. copticum*) در مقایسه با دیگر گونه های دارویی تحمل به خشکی بیشتری نشان داد.