



Soaking seeds of winter rapeseed with Quizalofop-P-Ethyl alters plant growth and improves yield in a rice-rapeseed cropping system



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ABSTRACT

Intercropping is an effective strategy to maximize production potential, profits, and land use efficiency. Sowing winter rapeseed in rice fields is a common cultivation technique in the Yangtze River basin in China, which is the world's largest region for rapeseed production. However, the 10–15 days crop overlap in rapeseed-rice cultivation results in low and unstable yields. In this study, we investigated, using both pot and field experiments, whether presoaking oil rape seeds with Quizalofop-*p*-ethyl (QPE) improved winter hardiness and yield. For the field trials, rapeseed was cultivated during two growing seasons from 2009 to 2011, in no-tillage fallow rice fields at the experimental site of Wuhan. Without QPE application, seedling quality was low for rapeseed sown in pots, and grown in shady conditions, or in the fallow rice field. Seedlings showed excessive elongation of the crown, resulting in low yields and yield-related traits at maturity. Soaking seeds in QPE improved the growth and survival of rapeseed seedlings, and improved their length, weight, and root neck diameter traits. Furthermore, the treatment decreased crown length during winter. Overall yield and related traits were enhanced by the QPE treatment. The most effective concentration of QPE was 120 ppm. Storing seeds for 2–10 months after soaking in QPE reduced any inhibitory effect of the treatment on germination rate and plant height. Our analyses demonstrated that treating seeds with QPE (120 ppm) increased the yield of rapeseed in a rice-relay cropping system.

1. Introduction

The global demand for vegetable oil production is increasing because of increased domestic use as edible oil, as well as its applications as a biofuel and forage crop (Finley, 2012). China is the second largest producer of rapeseed after Canada, with 11.6 million tons annually (FAOSTAT, 2015); nevertheless, the demand for vegetable oil and protein meal continues to increase the import of rapeseed oil into China (USDA, 2015). Rapeseed production has not yet fully reached its saturation level in China and is capable of satisfying more of the domestic demand (Wang et al., 2007).

China has two rapeseed oil production regions, a winter and a spring region. The winter region represents more than 90% of the total rapeseed planting area in China, with the largest rapeseed production belt being situated in the Yangtze River basin (Ren et al., 2015). Here, winter rapeseed is sown after rice or cotton, as a double- or triple-cropping system. The recommended planting time of rapeseed in this region is from late September to mid-October. However, because the rice is harvested in October, rapeseed planting is normally delayed until November (Wang et al., 2007). Rice-rapeseed intercropping is a good

way to use rice fallow land in a rice-rice-rapeseed multiple cropping system.

Multiple cropping is an efficient agronomic practice, which, if utilized correctly, can contribute significantly to increased crop production due to its substantial yield advantage compared to sole cropping. Intercropping can increase yields, utilize available resources and nutrients more efficiently, reduce weed, pest, and disease problems, and provide greater biological and economic stability. Therefore, the use of mono-crop production systems is decreasing, and intercropping rapeseed with rice, cotton, and wheat is becoming more common (Knörzer et al., 2009).

Relay intercropping is a beneficial practice to allow a multiple cropping pattern by resolving the timing conflicts for planting of various crops during the available growing season. It achieves this by the planting of the second crop (rapeseed) when the first crop (rice) is at the reproductive stage prior to its time of harvesting (Tanveer et al., 2017). Although relay cropping has the potential to increase production and crop yield following timely sowing of rapeseed, the direct seeding of rapeseed into rice field prior to harvesting of the rice can result in elongation of seedlings. This occurs because of insufficient light to

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young rapeseed plants due to shading by the standing rice crop. As a consequence, the rapeseed plants may not develop sufficient reserves for over-wintering. For optimal winter survival, the crown of rosette stage plants should be close to the soil surface with the height of the apical bud not exceeding 3 cm; they need to develop 6–8 leaves and have a root neck diameter of > 8 mm (Velicka et al., 2010). Stem elongation at this stage increases the risk of disease and weed infestation, leading to lower yields and oil contents (Su et al., 2014). As a result, only double cropping of rice is practiced in the Yangtze River belt area and farmers leave their land fallow after the winter rice crop, because survival and yield is not good for overwintered rapeseed.

The application of growth regulators is a common approach to manipulate rapeseed growth and to aid the development of winter resistance (Riffkin et al., 2012). However, foliar application of growth regulators is not suitable for relay-cropped rapeseed, because rapeseed plants develop large shoots before the previous crop has been harvested. An alternative approach is to treat seeds before planting; such treatment might inhibit rapeseed crown elongation, encourage winter hardiness, and subsequently improve plant survival and yield. Low doses of some herbicides can be used to modify plant growth (Velini et al., 2010). One such herbicide is quizalofop-*p*-ethyl (QPE), which is an ethyl derivative of quizalofop and has the IUPAC name ethyl (2R)-2-propanoate (EFSA, 2009; Mantzos et al., 2016). QPE is an effective herbicide that can be used to selectively control grass weeds in many broadleaf crops such as rapeseed, soybean, cotton, and sugar beet (Mahakavi et al., 2014). Quizalofop inhibits fatty acid synthesis by blocking the acetyl-Co-A carboxylase enzyme (Tang et al., 2014). Furthermore, it has a low risk of contaminating adjacent water resources because of its short half-life (0.5–0.7 days), and does not penetrate more than 10 cm into the soil. In addition, it shows limited transfer from broad-leaved plants such as rapeseed and sunflower (Guan and Zhang, 2013; Mantzos et al., 2016). The overall aim of our studies is to identify ways for improving rapeseed production in otherwise fallow fields during winter. As part of this objective, we investigated how QPE application affected winter rapeseed productivity by studying how treatment affected seedling emergence rate, survival rate, plant height, and winter growth, as well as other yield traits.

2. Materials and methods

2.1. Experimental materials and design

Overall, four pot experiments and two field trials were conducted during the rapeseed growing seasons from 2009 to 2011, at the Huazhong Agricultural University, Wuhan, China.

2.1.1. Identification of appropriate QPE concentrations

The pot experiments were carried out using rapeseed (*Brassica napus* L.) ‘Huashuang 5’. First, 20 g of seeds were soaked in 50 ml QPE at

concentrations of 40, 80, 120, 160, and 200 ppm for 12 h. As a control (0), seeds were soaked in 50 ml of distilled water. We sowed 10 seeds into plastic pots (0.3 m height, 0.2 m width) filled with 5 kg of soil (mixed with sand in a 3:1 ratio) and 2 g of nitrogen, phosphorous, and potassium (15% each) fertilizer. Each pot was shaded with a double layered net for 7 days. Each treatment was replicated four times. After the shading treatment, seedling germination rate, plant height, and root length were measured.

2.1.2. Identification of the appropriate QPE volume

Rape seeds (20 g) were soaked for 12 h in 10, 25 (complete immersion), 40, 55, and 70 ml of QPE solution (120 ppm); these treatments represented 2:4, 5:4, 8:4, 11:4, and 18:4 liquid-seed ratios respectively. As a control, 25 ml of distilled water was used (5:4 seed ratio). Subsequently, 10 seeds were sown in pots as described above, and kept in shaded conditions for 7 days. This experiment was replicated 4 times. Germination rate, seedling height, and root length were measured.

2.1.3. Effects of seed storage time

Seeds (20 g) were soaked in 50 ml of QPE (120 ppm) for 12 h. The seeds were then dried at room temperature, and stored for 0, 2, 4, 6, 8, and 10 months. After storage, 10 seeds were planted per pot (as described above), with four replicates per storage time. Seeds that had not been soaked in QPE or distilled water were used as the control treatment. Seven days after germination, seedling height and root length were recorded.

2.1.4. Effects of QPE on rapeseed growth in the shade

A pot experiment was conducted to determine the effect of shading on seedlings and mature plant stages. Seeds were soaked in QPE (120 ppm) for 12 h. As a control, seeds were soaked in distilled water. On October 15, 2009, seeds of the rapeseed cultivar ‘Huashuang 5’ were sown in pots placed in the shade for 10 or 15 days. After the shading treatment, they were thinned to two seedlings per pot at the 3-leaf stage. One of the plants per pot was harvested on December 22, 2009 to measure seedling growth parameters. Crown diameter was measured using the maximum value of the diameter of the rosette plant with open leaves. Root neck diameter was measured at the cotyledonary scar. The other plant was harvested at maturity on May 15, 2010 to measure yield-related traits.

2.1.5. Effect of QPE seed soaking in field trials

After selecting the appropriate concentration (120 ppm) and volume of QPE (25 ml for 20 g seeds) for seed soaking, as well as the optimal seed storage time, the rapeseed cultivar ‘Huashuang 5’ was sown in a fallow zero-tillage rice field in winter of 2009–2010 and 2010–2011, at Huazhong Agricultural University, Wuhan. Total precipitation (mm per month) and average temperature (°C) data were

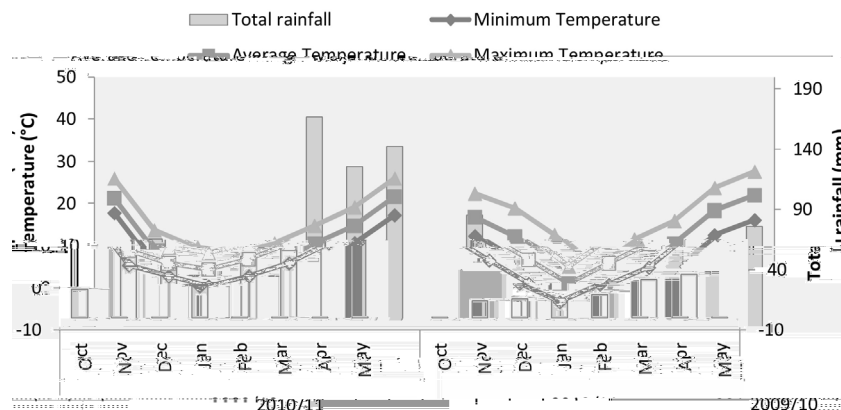


Fig. 1. Monthly total rainfall and monthly temperature during the crop growing season in the experiment conducted at Huazhong Agricultural University Wuhan, during 2009–2011.

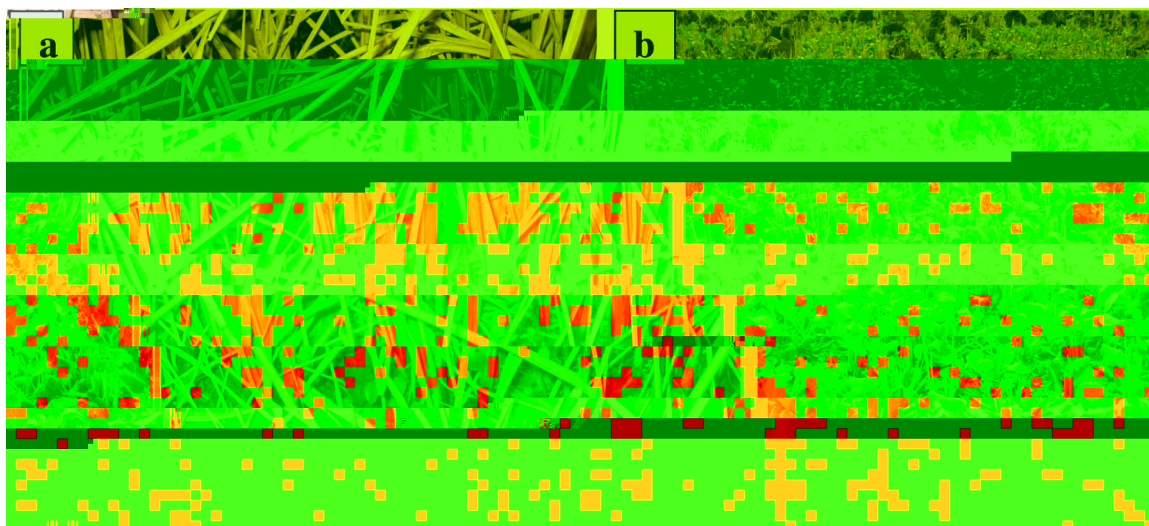


Fig. 2. Pictorial view of a rapeseed as relay crop in rice field, a) before rice harvest, and b) after rice harvest.

obtained from a nearby weather station (Fig. 1). Before sowing, the seeds were soaked for 12 h in 120 ppm QPE at a 5:4 liquid seed ratio (20 g seeds in 25 ml QPE) or in distilled water (control). The seeds were sown on 20 October at a rate of 6.0 kg ha^{-1} . The period of overlap between the rice and rapeseed in each plot lasted 10 days, after which rice crop was harvested (Fig. 2). Before sowing, single dose fertilizers were applied as P_2O_5 (12 g m^{-2}) and K_2O (9 g m^{-2}). Additionally, 22.5 g m^{-2} nitrogen was applied in the form of urea on November 10, and 11.25 g m^{-2} was applied on January 25, 2009 and 2010. Germination rates of the seedlings was recorded 15 days after sowing. Plant survival (for plants with more than 3 leaves) and wintering stage growth data was recorded on December 22 in both years. Twenty plants were uprooted per plot in order to measure their height, root neck diameter, length, number of leaves, shoot and root weight, leaf area index, and root to shoot ratio. On May 2, 2010 and 2011, when the plants were mature, 30 plants were randomly selected to measure height, number of branches, pods per plant, grains per pod, and 1000-grain weight. Each plot was harvested separately to determine the total yield.

2.2. Data analysis

All pot experiments followed a completely randomized design (CRD), and the field trial followed a randomized complete block design (RCBD). Analyses of variance and LSD analyses were used to identify whether treatments and interactions were significant, and whether the differences among the treatments were significant at the $P < 0.05$ level, using SAS 8.1 (SAS Corp., USA).

3. Results

3.1. Optimal QPE concentration

After soaking seeds in QPE concentrations from 40 to 200 ppm, germination rates of approximately 85% were found for all treatments and the control; no significant differences were present among the different treatments (Fig. 3). This suggests that the tested concentrations of QPE did not have an inhibitory effect on seed germination. However, seedlings that developed from seeds treated with QPE at concentrations from 80 to 200 ppm showed significantly decreased height after being shaded. Moreover, this effect became more apparent at higher concentrations. QPE also inhibited seedling root growth, although there was little difference between seedlings in the 40–120 ppm QPE treatments compared to the control. These results

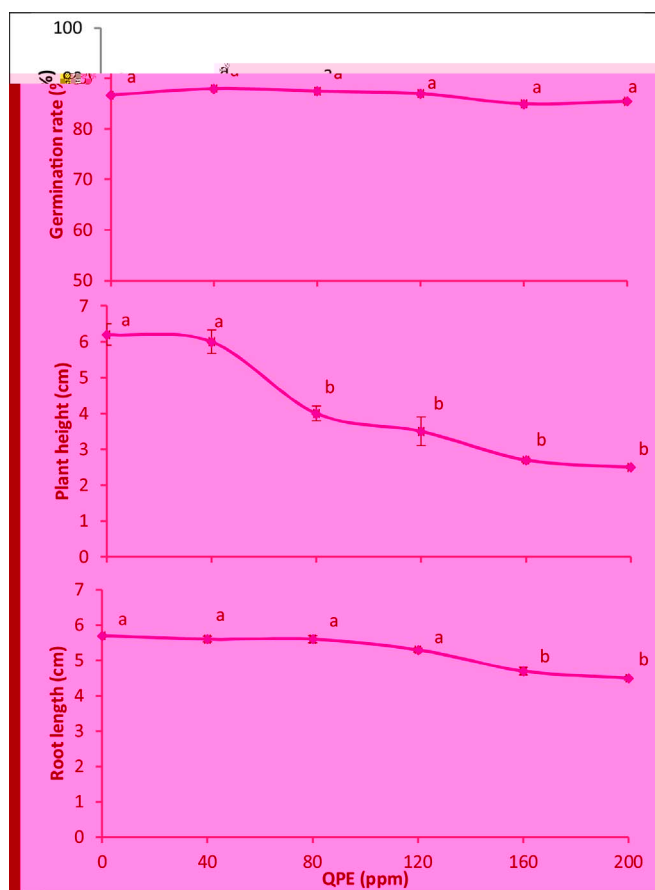


Fig. 3. Effect of seeds soaking treatment with different concentrations of quizalofop-ethyl (QPE) on the germination rate, seedling height and root length of rapeseeds. Different lettering denote the difference between the treatments at 0.05 probability level.

indicated that 120 ppm QPE was an appropriate concentration for soaking seeds as it reduced plant height but had no significant effect on germination rate or root length.

3.2. Effects of different liquid-seed ratios

Germination rate, plant height, and root length decreased when the amount of QPE increased (Fig. 4). The germination rate was only slightly decreased by an increase in QPE volume from 10 to 70 ml,

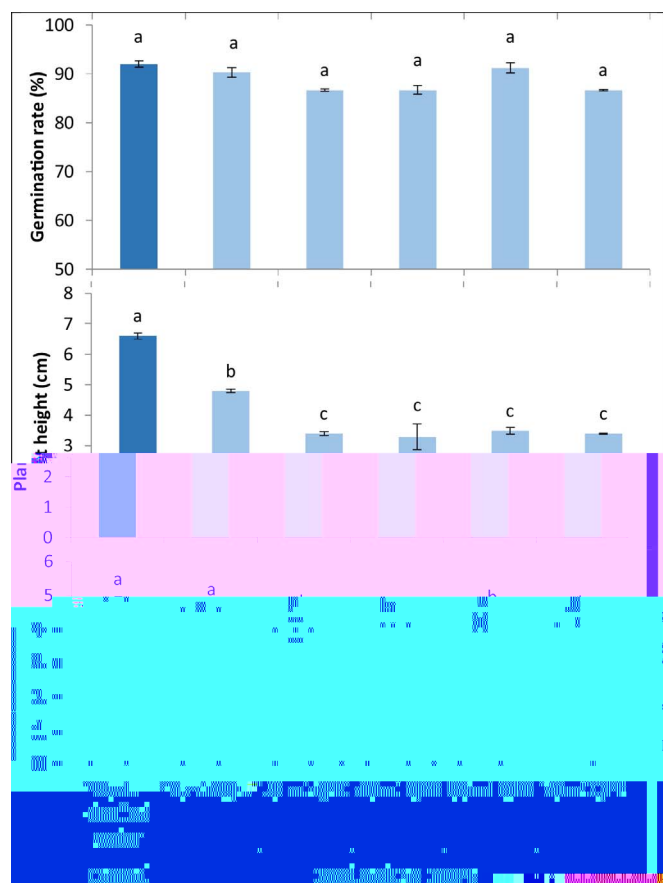


Fig. 4. Effects of seed soaking with distilled water (control) and different volumes of 120 ppm of quizalofop-*p*-ethyl (QPE) on germination rate, seedling height and root length of rapeseed. Different letters on columns indicate statistically significant differences among treatments at the $p < 0.05$ based on LSD tests.

compared to the control. Although seedling height was greatly inhibited at higher liquid-to-seed ratios, with a value of 4.8 cm at a 3:4 ratio and 3.4 cm at a 14:4 ratio. A similar trend was recorded for root length at increasing liquid-to-seed ratios. The reductions in plant height and root length were less obvious when the ratio was increased above 5:4 (completely immersed seeds), which indicates that higher ratios resulted in a weaker inhibitory effect.

3.3. Effects of seed storage time

Seed storage after soaking in QPE reduced the inhibition of seedling shoot and root growth, as compared to zero storage time (Fig. 5). Overall, the longer the period of storage after the QPE treatment, the smaller was the effect of the treatment on seedling growth. However, for germination rates, there was no significant effect of storage time.

3.4. Effects of QPE seed soaking on seedling growth and yield of rapeseed under shading conditions

Germination rates were not influenced by the different shading treatments of QPE-soaked seeds. However, prolonged shading did cause higher seedling death rates, a decrease in seedling height, fewer leaves, a thinner crown diameter and root neck diameter with substantial differences among the treatments, whereas the crown length was significantly increased (Table 1). Under the same shading conditions, soaking seeds significantly improved seedling quality through a higher germination rate, increased seedling survival rate, increased seedling height, higher crown thickness, more leaves, a higher crown diameter, and a notable decrease in the crown length.

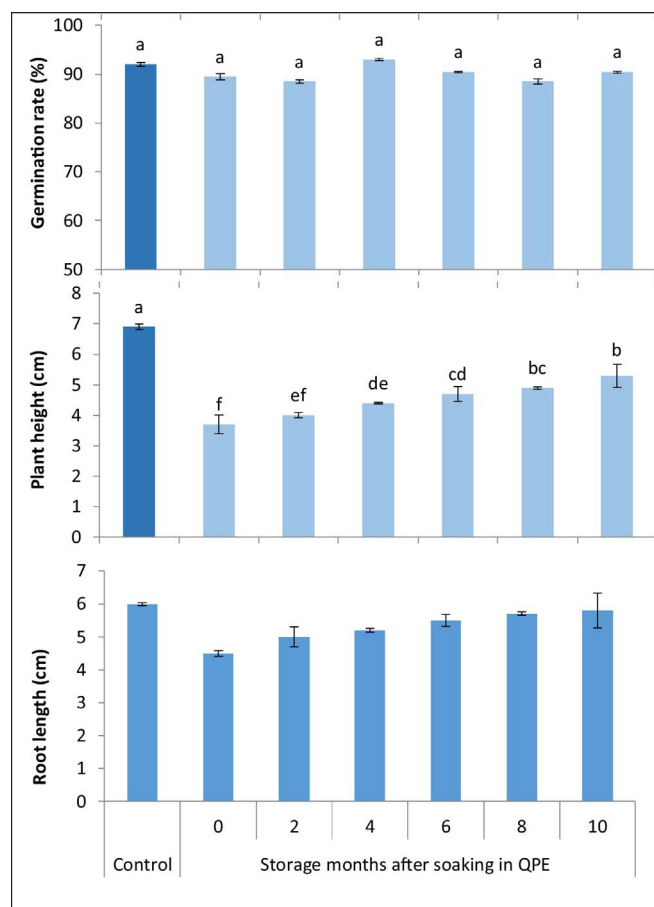


Fig. 5. Effects of different storage times after the seed soaking treatment with quizalofop-*p*-ethyl (QPE) on germination rate, plant height and root length of rapeseed. Control seeds were not soaked. Different letters on columns indicate statistically significant differences among treatments at the $p < 0.05$ based on LSD tests.

Shading time influenced yield traits and the overall rapeseed yield (Table 2). Increasing the shading time from 0 to 10 or 15 days in the same soaking treatment resulted in gradual increases in crown length and the number of seeds per pod. In contrast, seedling height, root neck diameter, shoot weight, root weight, root-to-shoot ratio, the number of pods, and 1000-seed weight declined with increased shading time. The QPE soaking treatment under the same shading conditions increased plant height, root neck diameter, shoot weight, root-to-shoot ratio, number of pods, and the 1000-seed weight, whereas the only trait that showed a reduction was crown length. Seeds soaked in QPE showed increased yield per plant compared to the control. An increase of 1.1 g per plant for un-shaded seedlings, 2.1 g per plant for those shaded for 10 days, and 2.7 g per plant for those shaded for 15 days was found compared to the control. Other traits changed in a similar manner. Overall, QPE had a greater influence on improving traits in seedlings and in maturing plants.

3.5. Effect of QPE seed soaking on seedling growth and yield of rapeseed cultivated in rice fallow field

Analysis of rapeseed grown in a fallow rice field showed that the different soaking treatments resulted in differences in emergence rates and seedling survival (Table 3). Emergence rate and seedling survival were highest for QPE treated seeds and lowest in the control during both years. Other indicators such as seedling height, leaf number, leaf area index, shoot weight, root length, and root neck diameter were also higher for QPE treated seeds.

In mature stages, all yield-related traits showed notable differences

among the control and QPE treatments in both years (Table 4). For QPE-soaked seeds, plant height, number of branches, and pods per plant were higher than those of the control plants during both years of the trial. Grains per pod and the 1000-grain weight were lower in the QPE treatments, which caused a slight reduction of yield per plant. As the density of mature QPE-treated rapeseed was significantly higher than that of the control plants, with an increase of 19 and 21.7%, the total yield was 23% higher in the winter of 2009–2010, and 21% higher in the winter of 2010–2011, respectively.

4. Discussion

Cultivating rapeseed in rice fields during winter is a very effective way to make use of otherwise fallow fields in triple-cropping rice and cotton areas, and can increase the multiple-cropping index. However, one constraint for production is the 10–15 days overlap between the two crops, during which the rapeseed is sown and the rice is harvested. Sowing rapeseed in a rice field at its reproductive stage (relay cropping)

tends to result in the elongation of the apical bud of rapeseed seedlings, reduction in the root neck diameter, and a decreased rate of survival, leading to a poor overall quality of rapeseed seedlings. If rapeseed seedling growth does not reach the optimum rosette stage before winter starts, the plants are unable to survive in the cold weather conditions, resulting in low and unstable yields (Velicka et al., 2012). One of our pot experiments showed that shading for 10 or 15 days could cause longer and thinner crowns in the seedlings, and a lower yield per plant (Tables 2 and 3). Thinner and longer crowns reduced the overwintering capacity of the plants, and the rosette biometric parameters are therefore important traits that determine the overwintering efficiency. Rapeseed overwinters well when the rosettes have 6–8 leaves, apical bud exceeds 3 cm, and the root neck diameter is 8–10 mm (Velicka et al., 2011). Velicka et al. (2012) additionally reported that rapeseed plants from later sowings had particularly thin root neck diameter compared to plants from earlier sowings; these poorer qualities caused late sown plants to have a lower winter survival rate.

One possible approach is to treat seeds with growth regulators or

inhibitors before sowing. This can potentially improve the yield of rapeseed in a triple-cropping system by inhibiting rapeseed crown elongation, increasing root neck diameter, and increasing seedling survival rates.

Rapeseed plants have a complex phenotype owing to their indeterminate and dynamic growth and rosette structure. Inhibiting factors for late sown rapeseed in the field include shading due to late sowing, and competition from neighboring plants. Therefore, the rapeseed competition strategy involves a reallocation of growth resources to expand the leaf area and elongate the stem. However, this strategy likely causes a reduced seed yield. The stem elongation strategy can be prevented by applying growth retardants (Kurepin et al., 2013).

QPE inhibited crown elongation without having a significant effect on seedling emergence. One hypothesis for this pattern of response is that growth regulators influence plant growth during the post-germination stage, whereas the seed coats acts as a carrier for such growth regulators (Pasian and Bennett, 2001). Herbicides can be applied to crops at low rates in order to regulate their growth (Velini et al., 2010). To our knowledge, no study has focused on the effects of quizalofop-like herbicides as a pre-sowing seed treatment. The toxicity of QPE, when applied to soils or plant leaves, is well documented. According to Mahakavi et al. (2014), QPE applied to soils at low concentrations (0.5%) stimulate growth performance in blackgram (*Vigna mungo*). However, it reduces plant height, chlorophyll levels, and photosynthesis rate at subsequent applications with increased concentrations of QPE. Quizalofop is used to control annual and perennial grasses, and works by inhibiting fatty acid biosynthesis through inactivation of acetyl-CoA carboxylase. However, in broad-leaved plants, the absorption of QPE is limited, and most of the chemical remains at the site of application with limited translocation through the phloem or via the transpiration stream (Hutson, 1998).

In the present study, seed soaking with QPE improved the rapeseed seedlings after sowing in winter, and raised yields per plant at maturity during both years. These effects might be more prominent with prolonged shading time. Furthermore, minimum plant height and minimum number of branches were observed in control plants. This effect is probably caused by the relatively late timing of planting, which

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