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Soaking seeds with paclobutrazol enhances winter survival and yield of rapeseed in a rice-rapeseed relay cropping system

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Abstract

Rice-rapeseed relay cropping is an effective way to use winter fallow fields and increase land use efficiency. However, the overlapping period of 10–15 days for rice-rapeseed cultivation results in reduced winter survival and less yields. Therefore, in this study, we investigated if the treatment of oil rape seeds with paclobutrazol (PAC) improves its winter hardiness and yields by altering its crop morphology. First, a pot experiment was conducted to determine the appropriate PAC concentration to soak seeds upon seedling growth and the germination rate. Another pot trial was performed to check the responses of different rapeseed cultivars to the proposed treatment. Rapeseed was cultivated in no-tillage rice fields at an experimental site in Wuhan from 2009 to 2011. In the absence of PAC treatment, rapeseed sown in pots and rice fields showed excessive elongation of the crown, which resulted in low yields and yield-related traits at maturity. Soaking seeds in PAC improved seedling growth, seedling survival and crown diameter, but decreased the crown length during winter. The most effective concentration of PAC was 4 mg/L and higher concentrations significantly reduced seed germination rates. Our results demonstrated that rapeseed sown in rice fields after the seeds had been soaked with PAC improved seedling quality at the wintering stage and yield at maturity.

Keywords: *Brassica napus*; Relay cropping; No-tillage rice field; PAC; Yield improvement.

Introduction

Sustainable use of natural resources is critically important to ensure global food security. The increasing population density and growing demands for food, feed and bioenergy are increasing pressure on agricultural land. On the other hand, depletion of natural resources, climate change and soil degradation, are limiting the suitability of land for agriculture and cultivation practices. Resource-use efficiency is an important aspect for considering the suitability of a cropping system (Fan et al., 2011; Tanveer et al., 2017). Crop rotation is an effective method to reduce soil erosion and weed infestation, as well as to enhance land use efficiency (Rathke et al., 2005; Yamane et al., 2016).

Rapeseed (*Brassica napus* L.), an important agricultural crop, is a major source of vegetable oil and fodder and is also a potential biofuel crop (Finley, 2012). China is the second largest producer of rapeseed after Canada, with a production of 11.6 million tons (FAOSTAT, 2015). The Yangtze River Basin in China is the world's largest belt for rapeseed production (Khan et al., 2017) contributing 91% of the total rapeseed and 70%

of the total rice production (Yousaf et al., 2017). Yangtze River Basin is a major planting area for the rice-oilseed rape rotation system, where winter rapeseed is sown in crop rotation with rice or cotton, under a double- or triple-cropping system (Hu et al., 2017; Ren et al., 2015). The rape-rice-rice triple cropping system accounts for 40% of the total rapeseed planting area in the middle and lower reaches of the Yangtze River (Wang et al., 2007). In rice-rice-rapeseed cropping system after the harvest of early rice in July, late rice is transplanted in July and harvested in October. After which rapeseed are sown or transplanted in the paddy fields (Zhang et al., 2014). The only constraint affecting high yield of rapeseed in the rice-rice-rapeseed triple cropping system is the late sowing of rapeseed due to the overlap of rapeseed sowing and late rice harvesting dates (Wang et al., 2011).

Relay cropping is a method of overlapping intercropping in which succeeding crop is planted into standing first crop after the first crop has reached at reproductive stage (Tanveer et al., 2017). Relay cropping of rapeseed in the standing rice crop before harvest is an effective way to reduce the cost of land preparation and facilitates timely planting of rapeseed. Furthermore, adopting zero tillage of rapeseed as relay crop also conserves soil moisture, protects soil from erosion and reduces tillage cost (Tan et al., 2014; Zhang et al., 2014). Seedling survival during winter is one of the key factors for successful growing of winter oilseed rape. However, sowing of rapeseed in rice field results in crown elongation due to insufficient light caused by shading from standing rice crop (Anwar et al., 2017); this in turn results in insufficient reserves for over-wintering of rapeseed plants and increases the risks of diseases, weed infestations and lodging, leading to lower yield and oil content (Su et al., 2014).

The use of foliarly applied plant growth regulators is a common approach to manipulate the growth of rapeseed and also to develop winter resistance in this crop (Riffkin et al., 2012). However, foliar application of growth regulators in relay cropped rape fields might not be effective owing to the elongated hypocotyls in rapeseed seedlings. Seed treatment before sowing could be an effective option to inhibit crown elongation and prepare seedlings for wintering, which subsequently improves seedling survival and yield. Paclobutrazol (PAC) is a type II growth regulator of the triazole group, which works by inhibiting gibberellic acid (GA) synthesis. Currently, PAC is widely used in combination with foliar and soil application methods, to minimize the adverse effects of abiotic stresses such as drought (Shahrokhi et al., 2011) and salinity (Sharma et al., 2011) and to enhance transplantation tolerance (Souza-Machado et al., 1998). The objectives of this study were to manipulate the rape relay cropping system in no-till winter fallow fields using the PAC seed soaking treatment and to examine its effects on seedling emergence and survival rate, crown length reduction, winter growth, as well as yield traits of rapeseed.

Materials and Methods

Experiment materials and design

Overall two pots and two field trials were performed from 2009 to 2011 during the rapeseed growing seasons at the Huazhong Agricultural University, Wuhan, China.

Identification for the appropriate PAC concentrations under shading condition

The preliminary pot experiment was conducted with rapeseed (*Brassica napus* L.) line Huashuang 5. For this 20 g of seeds were soaked in 50 ml of paclobutrazol (PAC) solutions at different concentrations (0, 2, 4, 6, 8, 10 and 20 mg/L). After soaking for 12 hours, 10 seeds were sown in plastic pots (0.3 × 0.2 m, height and width) containing 3 kg of soil and sand mixture (3:1, W/W). Before sowing, the field capacity of the pots was maintained. Each treatment was replicated four times. Fertilizer, 2 g containing nitrogen, phosphorous and potassium (15% each) were applied at sowing. Pots were shaded with a double layered net for 7 days. After the shading treatment, the seedling germination rate, height and root length were measured.

Screening rapeseed cultivars

From the pot experiment above, the PAC level (8 mg/L) at which 50% reduction in germination rate took place was selected to test the growth of the rapeseed lines. The seeds (20 g) of eight winter rapeseed lines, Huangza 13, Zhongza 11, Zhongza 12, Zhongza 7819, Zhongshuang 7, Zhongshuang 9, Zhongshuang 10 and Huashuang 5, were soaked in 50 ml of PAC solution (8 mg/L) for 12 hours and ten seeds were sown per pot. Each treatment was repeated four times and pots were subsequently placed in the shade for 7 days. At the end of the experiment, seedling germination rate and plant height were measured.

Effects of PAC seed soaking in field trials

Rapeseed cultivar Huashuang 5 was sown in a zero-tillage rice field during the 2009-2010 and 2010-2011 rapeseed winter sowing seasons at Huazhong Agricultural University, Wuhan. Soil samples were collected from the research field at a depth of 0–30 cm before sowing, sieved and analyzed in the laboratory using standard techniques. Physicochemical properties of the soil are presented in Table 1. Soil properties remained almost same before rapeseed sowing in both years. Temperature and precipitation data was collected from the nearby weather station (Figure 1). Before sowing, the seeds were soaked for 12 hours in 4 mg/L of PAC and sown on 25 October. Rice field was drained 1 week before rapeseed plantation. The seeds of rapeseed were sown broadcast at a seeding rate of 4.0-4.5 kg ha^{-1} between the rows of rice. The period of overlap between the rice and rapeseed crops in field lasted 10 days after which rice crop was harvested 15-20 cm above the ground level (Figure 2).

Table 1. Soil conditions of trial sites during the rapeseed growing season in 2009-2010 and 2010-2011.

Trial site	pH	Organic matter (g kg^{-1})	Available N (mg kg^{-1})	Available P (mg kg^{-1})	Available K (mg kg^{-1})
2009/10	6.3	37	111	9.8	105
2010/11	6.1	35	108	8.2	104

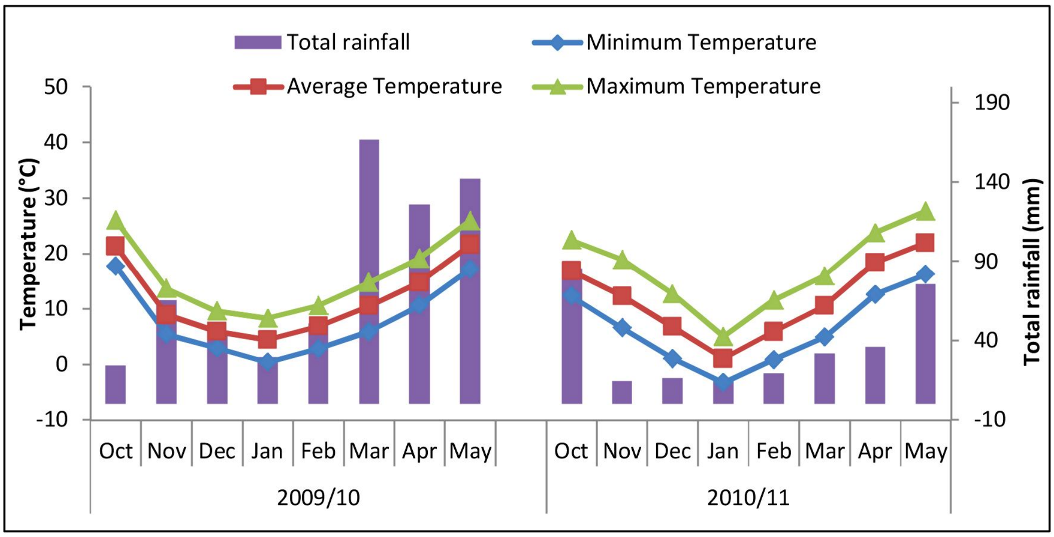

Figure 1. Meteorological conditions of site during two stages of rapeseed growth.

Figure 2. Pictorial view of rapeseed as relay crop in rice field after rice harvesting.

Single dose of phosphorous (P_2O_5, 12 g m^{-2}) and potassium (K_2O, 9 g m^{-2}) were applied before sowing. Nitrogen was applied in the form of urea with 22.5 g m^{-2} before sowing and 11.25 g m^{-2} was applied at bud initiation stage. Germination rates of the seedlings were recorded from 1 m^2 plot area 15 days after sowing, whereas seedling survival rate was recorded at 2-3 leaves stage. On 20 December, 20 plants per plot were uprooted to measure the height, crown diameter, number of leaves, shoot and root weight, leave area index and root to shoot ratio. Plant height was measured from cotyledon scar up to the highest bud. Root neck diameter was measured under the cotyledon scar using Vernier calipers. Crown length was measured from soil surface to base of the crown.

At maturity 20 plants were randomly selected to measure their height, number of branches, pods per plant, grains per pod and their 1000-grain weight. The remaining plants from each plot were harvested to determine the total seed yield (t ha^{-1}). During experiment to control weeds, 10% quizalofop-p-ethyl (0.6-0.8 ml/ L^{-1} water) was sprayed at 2-4 leaves stage and 50% benazolin (0.6-0.8 ml/ L^{-1} water) was sprayed at 5-6 leaves stage. To control aphid, 50% Imidacloprid, (0.8-1 ml/ L^{-1} water) was sprayed when more than 8% of plants were infested with aphids.

Statistical analysis

Pots experiments were arranged in a completely randomized design with four replications and the field trial with randomized complete block design with three replicates per treatment. Analyses of variance and LSD analyses were used to check the significant influence and difference among treatments and their interactions at a $P < 0.05$ level, using SAS 8.1 (SAS Corp., USA).

Results

Screening for the PAC concentrations

After soaking seeds in PAC treatment (2 to 20 mg/L), germination rates dropped significantly for the seedlings which receive concentrations above 4 mg/L, as compared to control after being shaded (Figure 3). The lower concentrations (2 and 4 mg/L) were significantly non-different from the control. Whereas, 52 % reduction of germination rate was observed at 8 mg/L. Maximum reduction in germination rate (84%) was recorded at the highest PAC level (20 mg/L). PAC also inhibited the seedling shoot and root growth (Figure 4). Significant reduction of shoot length occurred at PAC concentration above 4 mg/L. Lower concentrations (2 and 4 mg/L) showed non-significant difference from control. Root length was also reduced at and above 4 mg/L. These results indicated that the appropriate soaking concentration of PAC was 4 mg/L as it reduced plant height but had no significant effect on germination rate.

Screening for the rapeseed cultivars

The PAC concentration 8 mg/L, at which 50% reduction in germination rate was observed in first experiment, was used to observe the response of different rapeseed cultivars (Table 2). After a 10-days shading period, the germination rates of all cultivars were higher than 90% under control conditions. Seeds soaked with PAC showed inhibited germination rates and seedling height. Reduction in germination rate in all cultivar was ranging from 39% to 64%, with average decrease of 52%. PAC treatment caused an average decrease of 38% seedling height compared to the control. More reduction in germination rate was recorded in lines Zhongshuang 9, Zhongza 11 and Zhongshuang 11, whereas germination rate was least affected in Huaza 13 and Zhongza 7819 after PAC seed soaking and shading. Reduction in plant height was more (> 40%) in Huaza 13, Zhongshuang 7 and Zhongshuang 10 as compared to Zhongza11 and Zhongza 7819 (< 30%) after PAC seed soaking.

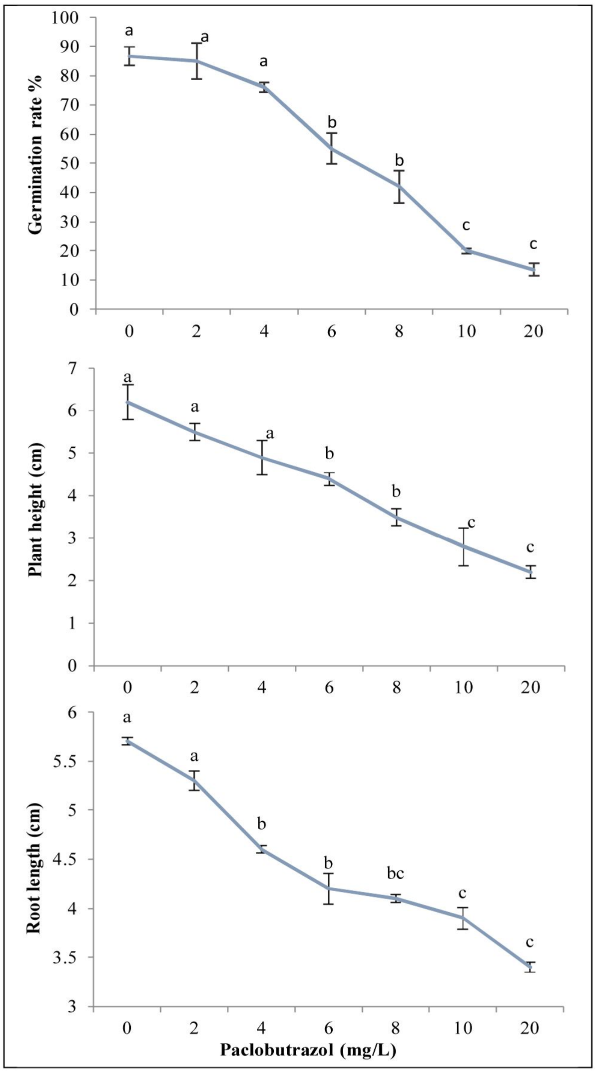

Figure 3. Effect of seed soaking in different concentrations of paclobutrazol on germination rate, plant height and root length of rapeseed (mean±S.E). Different letters denote the difference between the treatments at 0.05 probability level.

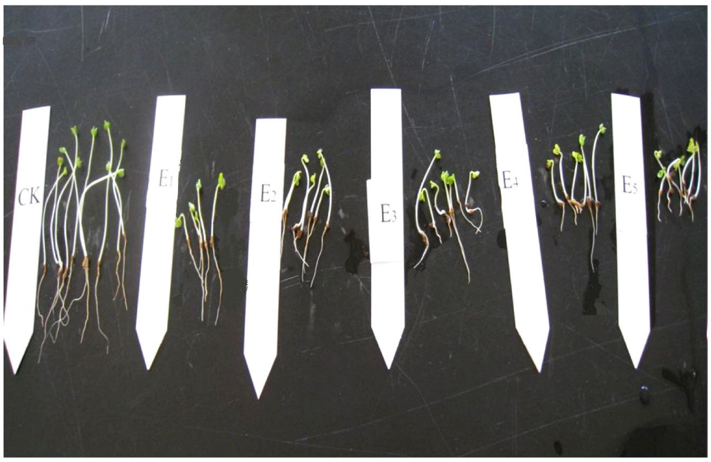

Figure 4. Growth of seedlings of rapeseed after seed soaking treatments with paclobutrazol. CK: control, E1-E5: 2, 4, 6, 8 and 10 mg/L Paclobutrazol.

Table 2. Cultivars screening after seed soaking in selected concentrations of paclobutrazol (PAC).

Lines	Germination rate			Plant height		
	Measured value (%)		Decrease (%)	Measured value (cm)		Decrease (%)
	Control	PAC	PAC	Control	PAC	PAC
Huaza 13	96	47	51.0**	5.1	2.5	51.0**
Zhongza 11	96	40	58.3**	5.5	3.8	30.0**
Zhongza 12	96	42	56.3**	5.9	3.5	40.7**
Zhongshuang 7	100	53	47.0**	5.3	3.0	43.4**
Zhongshuang 9	92	33	64.1**	4.9	3.2	34.7**
Zhongshuang 10	93	39	58.1**	4.8	2.8	41.7**
Huashuang 5	98	60	38.8**	7.1	4.5	36.6**
Zhongza 7819	94	57	39.4**	5.7	4.0	29.8**
Average	96	46	51.6 **	5.5	3.4	38.5 **
$LSD_{0.05}$	4	8	5.4	0.4	0.6	6.7
$LSD_{0.01}$	5	11	7.3	0.5	0.8	9.1

PAC level was 8 mg/L. * and ** indicate a significant difference at 0.01 and 0.05 probability levels, respectively. $LSD_{0.05}$ and $LSD_{0.01}$ denote the difference between different treatments at a 0.05 and 0.01 probability level, respectively.

Effect of seed soaking on seedling growth and yield of rapeseed in rice fallow field

Rapeseed cultivation in the fallow rice field showed that the soaking treatment lead to different seedling survival and emergence rates (Table 3). Seedling emergence rate was decreased, whereas seedling survival was improved significantly with PAC treatment during both years. At the wintering stage of rapeseed, other indicators such as seedling height, leaf area index, shoot weight, root weight, root length and root neck diameter were remarkably higher for the PAC treated seeds compared with the control (Table 3, Figure 5). PAC seed soaking significantly decreased the crown length at wintering stage, although number of leaves was not affected.

In mature stage, all yield related traits showed notable differences among the control and PAC treatments (Table 4). When seeds were soaked in PAC, plant height and pods per plant were higher than the control plants during both years of the trial, whereas numbers of branches were increased significantly from 4.1 to 5.1 during 2009-2010 trial but remain unaffected in next year. Grains per pod and the 1000-grain weight were lower in the PAC treated seeds, which caused a slight reduction of yield per plant in first year. Yield per plant showed 2% reduction in 2009-2010 trial in PAC soaked seeds as compared to control, whereas remain unaffected in the next year trial. The population density of mature PAC treated rapeseed was significantly higher than the control plants, which was increased from 57 plants m^{-2} to 63 plants m^{-2} in first year and from 53 plants m^{-2} to 58 plants m^{-2} in the second year trial. As the population density showed 10% increase, the total yields also rose with 11% increase in 2010 and 9% increase in 2011, respectively.

Table 3. Effects of paclobutrazol (PAC) soaked seeds on seedling survival and seedling traits of rapeseed cultivated in a fallow rice field site in winter 2009-2010 and winter 2010-2011.

Trial Season	Treatment	Seedling survival		Seedling traits at wintering stage							
		Seedling emergence (%)	Seedling survival (%)	Plant height (cm)	No. of green leaves	LAI	Shoot weight (g)	Root weight (g)	Root length (cm)	Crown length (cm)	Crown diameter (cm)
2009/10	Control	68.7	58.2	19.4	5.2	1.89	0.42	0.065	5.9	4.8	1.80
	PAC	45.5	70.4	20.3	5.4	2.26	0.56	0.071	7.2	3.3	2.35
2010/11	Control	64.1	61.2	9.7	5.0	1.09	0.27	0.045	2.0	4.7	1.80
	PAC	42.5	69.5	12.1	5.2	1.83	0.37	0.051	3.3	3.9	2.20
$LSD_{0.05}$		10.7	9.4	1.8	0.4	0.31	0.11	0.006	1.1	0.8	0.45
$LSD_{0.01}$		15.0	13.2	2.5	0.6	0.43	0.15	0.008	1.5	1.1	0.63

PAC level was 4 mg/L. $LSD_{0.05}$ and $LSD_{0.01}$ denote the difference between the treatments at a 0.05 and 0.01 probability level, respectively.

Table 4. Effect of Paclobutrazol (PAC) soaked seeds on the yield and yield traits of rapeseed cultivated in a fallow rice field in winter 2009-2010 and winter 2010-2011.

Trial Season	Treatment	Density (plants m^{-2})	Plant height (cm)	Number of branches ($plant^{-1}$)	Number of pods ($plant^{-1}$)	Grains per pod	1000-grain weight (g)	Yield per plant (g)	Total yield (t ha^{-1})
2009/10	Control	57.5	112.3	4.1	82.0	16.0	2.98	3.91	2.14
	PAC	63.1	119.3	5.1	94.8	14.4	2.81	3.84	2.37
2010/11	Control	53.2	103.8	4.7	71.7	15.1	4.01	4.34	2.22
	PAC	58.5	109.4	5.1	84.9	13.6	3.75	4.33	2.43
$LSD_{0.05}$		4.04	5.3	0.6	0.211	1.3	0.18	1.13	0.138
$LSD_{0.01}$		5.66	7.4	0.8	15.7	1.8	0.25	1.58	0.193

PAC level was 4 mg/L. $LSD_{0.05}$ and $LSD_{0.01}$ denote the difference between the treatments at a 0.05 and 0.01 probability level, respectively.

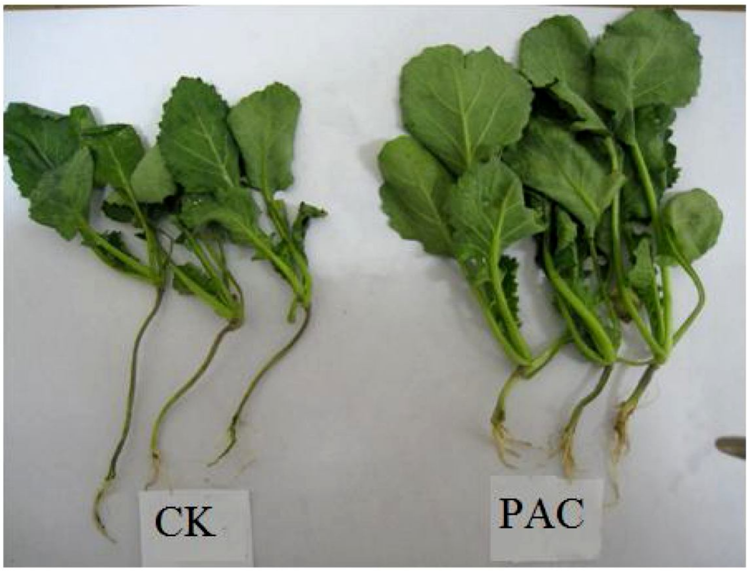

Figure 5. Seedlings of rapeseed cultivated in a fallow rice field, CK: control; PAC: paclobutrazol soaked seeds.

Discussion

The major constraint for the double and triple cropping of rice-winter rapeseed is the late sowing of rapeseed due to wet fallow field and overlap growth period of the both crops. Rapeseed plant has a complex growth pattern due to rosette structure and indeterminate growth. Relay cropping of winter rapeseed in standing rice crop resulted in over elongation of crown due to the shading effect by rice plants. Therefore, the plant reallocates the growth pattern like elongation of stem to reduce competition due to shading resulting in taller plants with less leaf area, a situation which is likely to cause reduced winter survival in winter rapeseed. The stem elongation strategy may be prevented by applying growth retardants that inhibit gibberellic acid (GA) biosynthesis (Kurepin et al., 2013).

In this study, rape seedling growing under shade was significantly inhibited by soaking the seeds in PAC, although seedling emergence rates also declined with this treatment. Bennett et al. (2004) reported that seedling height of marigold, cabbage and tomato could be controlled by soaking their seeds in PAC without affecting their seedling emergence. Nonetheless, this treatment reduced the seedling emergence of geraniums, kale and pepper. The inhibition of seedling germination might depend on the chemical nature of the seed coat, such as the location and type of a semi-permeable layer in the seed coat. PAC reportedly works by adsorbing to the seed coat, rather than by penetrating into the seed. After sowing, it subsequently diffuses into the growth media, from where it is absorbed by the roots (Pasian and Bennett, 2001). Growth regulators influence plant growth during the post-germination stage, whereas seed coats act as a carrier for such growth regulators (Pasian and Bennett, 2001). Gallardo et al. (2002) studied the proteomics of *Arabidopsis* seeds after incubating them in PAC. They found that PAC completely repressed the radical protrusion by inhibiting the gibberellin biosynthesis, whereas an addition of gibberellin acid to the germination medium reversed this inhibition. Gibberellins play a key role in the late stages of seed germination and they are involved in many germination processes before the radicle protrusion stage. PAC did not affect the initial mobilization of seed protein and lipid reserves, but it blocked the proteome in the germination mode, hindering the development of a radicle protrusion and thus affecting germination rate (Gallardo et al., 2002).

In this study, when seeds were soaked in PAC and the seedlings were growing in pots under shading conditions, plant height reduced gradually with increasing PAC concentrations (Figure 3). Low light or shading at early stages of plants stimulates the shade avoidance mechanisms like shoot elongation and upward positioning of leaves to capture maximum light (Rondanini et al., 2014) and also increases the leaf area with reduction in specific leaf weight (Brunel-Muguet et al., 2013). These mechanisms resulting in longer and thinner crown which ultimately lessen the overwintering efficiency and seedlings survival rate of rapeseed.

Rapeseed rosette traits are important parameters to attain the overwintering capacity. Rapeseed overwinters well if the crown length is more preferably less than 3 cm and root collar diameter is at least 8-10 mm (Velicka et al., 2006; Velicka et al., 2011). In the present trial, root crown length was almost 5 cm, which might be risky for good rape wintering according to Velicka et al. (2006). Root collar is the most vulnerable part of the rapeseed plant, which if reaches above the soil surface, is easily damaged by frost and the plants destroyed (Lääniste et al., 2007). Rapeseed plants with thinner root collars have lower winter survival rate (Velicka et al., 2012). In the present field experiment, the application of PAC significantly increased the root crown diameter whereas decreased the crown length and thus increased the seedling quality for overwintering. Moreover, the reductions in plant height were cultivar dependent (Table 2). Among the 8 studied cultivars, one showed a reduction that was higher than 50% at 8 mg/L of PAC.

In field trial plant height at wintering and maturity stage was more by PAC seed soaking as compared to control (Tables 3 and 4). This might be attributed to better wintering traits observed in PAC treated plants whereas control plants overwintered quite poorly because of overgrowing in autumn (Butkute et al., 2006). Previous study by Berova et al. (2002) indicated that wheat seedlings treated with PAC showed significant reduction in length and biomass but after chilling stress more height and fresh and dry biomass was recorded in PAC treated seeds. PAC ameliorated the deteriorative effects of stress by enhancing antioxidative system.

Results from the two field trials showed that rapeseed without PAC soaking treatment had less yields (Table 4). Rapeseed yield is generally influenced by the duration of vegetative growth period, starting of flowering, radiation use of the established stand and the availability of assimilates for pod setting and seed filling (Diepenbrock, 2000). Although rapeseed plants have a substantial capacity to compensate for damage, an optimal stand establishment before the onset of winter is a prerequisite for both high yields and high yield stability (Diepenbrock, 2000). According to Szczepanek et al. (2016), rapeseed plants produced higher yield when they overwinter well. Results from this trials indicate that soaking seeds in PAC improved the total yields (t ha^{-1}) of relay cropped rapeseed in rice field, which was 9-11% more as compared to control, whereas grains per pods was decreased during both years and yield per plant was 1.8% less in 2011. Therefore, increase in total yield is due to the improvement in wintering survival rate which also resulted in more planting density in PAC treated plants. Previous studies on the foliar application of PAC indicated positive effects on the yield through increasing resistance for lodging and pod shattering

(Kuai et al., 2015). Foliar PAC application also increased assimilate availability and translocation to the developing seeds. In general, PAC alters the sink strength within the plants, resulting in a redistribution of assimilates to the meristematic regions, other than the shoot apices and also improves the flow of assimilates towards the reproductive structures (Setia et al., 1996). Contrary results on PAC seed soaking of tomato and cucumber seeds indicated that the high soaking concentration of PAC (250-2000 mg/L) inhibited the flowering and fruit growth and ultimately caused reduction by inhibiting the seedling growth (Magnitskiy et al., 2006).

Thus, the seed soaking in PAC showed an improved seedling survival and more favorable agronomic traits such as plant height, LAI, shoot and root weights, crown diameter, number of leaves, branches, pods per plant and the total yield of rapeseed in rice fallow field.

Conclusion

Sowing rapeseed in rice fallow fields is an effective cropping system. Pot experiments and field trials both revealed that sowing rapeseed in rice field can lead to the over-elongation of seedling crowns by shading which results in weaker seedlings with decreased seedling survival, seedling conditions and yields. Treating seeds with PAC improved seedling quality by inhibiting crown elongation and increasing seedling survival rate in the winter. The most effective concentration of PAC was 4 mg/L and higher concentrations strongly inhibited seedling germination. Thus, soaking seeds with PAC could potentially improve seedling quality in winter fallow fields, increase seedling survival rate and enhance crop densities at harvest and the actual yield.

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