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2-Azahypoxanthine and imidazole-4-carboxamide produced by the fairy-ring-forming fungus increases wheat yield

Hiroyuki Tobina[a†], Jae-Hoon Choi[b†], Tatsuo Asai[a], Yoshikazu Kiriiwa[c], Tomohiro Asakawa[d], Toshiyuki Kan[d], Akio Morita[c,*], Hirokazu Kawagishi[b,c,e*]

[a] *Center for Education and Research in Field Sciences, Faculty of Agriculture, Shizuoka University, 63 Kariyado, Fujieda, Shizuoka 426-0001, Japan*

[b] *Research Institute of Green Science and Technology, Shizuoka University, 836 Ohya, Suruga-ku, Shizuoka 422-8529, Japan*

[c] *Graduate school of Agriculture, Shizuoka University, 836 Ohya, Suruga-ku, Shizuoka 422-8529, Japan*

[d] *School of Pharmaceutical Sciences, University of Shizuoka, 52-1 Yada, Suruga-ku, Shizuoka 422-8526, Japan*

[e] *Graduate School of Science and Technology, Shizuoka University, 836 Ohya, Suruga-ku, Shizuoka 422-8529, Japan*

*Corresponding authors. H.K., Tel.: +81 54 238 4885; fax: +81 54 238 4885. A.M., Tel.: +81 54 238 4891; fax: +81 54 238 4891.

E-mail addresses: dhtobin@ipc.shizuoka.ac.jp (H. Tobina), ajchoi@ipc.shizuoka.ac.jp (J.-H. Choi), atasai@ipc.shizuoka.ac.jp (T. Asai), akykiri@ipc.shizuoka.ac.jp (Y. Kiriiwa), t.asakawa@u-shizuoka-ken.ac.jp (T. Asakawa), kant@u-shizuoka-ken.ac.jp (T. Kan), aamorit@ipc.shizuoka.ac.jp (A. Morita), achkawa@ipc.shizuoka.ac.jp (H. Kawagishi)

[†] These authors contributed equally to this work.

Abstract: Two-year field examinations to see effects of the principle components of a fungus causing "fairy rings" phenomena on lawns, 2-azahypoxanthine (AHX) and imidazole-4-carboxamide (ICA), on wheat were performed. The treatment of AHX or ICA was performed, in the early (seedling treatment, seed soaking treatment) and late (field treatment) developmental stages, and the resulting number of ears and grain weight per plant increased. AHX treatment increased the number of ears before tillering and ICA treatment increased the number after tillering. The high temperature during tillering stage in 2011 was a stress condition for wheat production in fields. The results indicated that AHX and ICA increased number of tillers and then number of ears, resulting in the increase of the grain yield even though under high-temperature stress.

Key words: Fairy rings, Field examination, Imidazole-4-carboxamide, 2-Azahypoxanthine, Wheat, Yield increase.

1. Introduction

Rings or arcs of fungus-stimulated plant growth occur often on the floor of woodlands, in agricultural areas and in grasslands worldwide, which are commonly called "fairy rings". Usually, fruiting bodies of larger fungi appear on the rings after the stimulation. This phenomenon had been a mystery attributed to "fairies" before current study. Fifty-four species of fungi have been identified to be the cause of fairy rings and the most common fairy-ring-forming fungus in Japan is *Lepista sordida* (Komurasakishimeji in Japanese). Recently, two compounds, 2-azahypoxanthine (AHX) and imidazole-4-carboxamide (ICA), were identified as "fairies" (specific plant growth regulators) in the fungus (Fig.1; Choi et al., 2010a, 2010b). Furthermore, very recently, we proved the existence of endogenous AHX in plants (Choi et al., 2014).

When bentgrass (*Agrostis palustris* Huds.) and rice (*Oryza sativa* L.) were cultivated with AHX solution, shoot and root elongation of the seedlings were accelerated (Choi et al., 2010a). When rice and potato were cultivated with 5 and 50 μM AHX in pot experiment, the yield per plant increased by 25% and 19%, respectively (Choi et al., 2010a). Rice grain yield per plant increased by cultivation of the plant with 2 μM ICA in pot experiment (Choi et al., 2010b).

Oligo-DNA microarrays for rice seedlings treated by AHX revealed that genes responsible for tolerance to environmental stresses were induced (Choi et al., 2010a). In fact, when AHX-treated rice seedlings were cultivated under 0.1 M NaCl or 35°C, they showed tolerance to these stresses (Choi et al., 2010a). These results show that AHX and ICA could improve growth for crops under environmental stresses.

Wheat (*Triticum aestivum* L.) is widely cultivated in semiarid and cool areas, and its cultivated acreage is around 221 million hectare in the world (United States Department of Agriculture, 2012). Wheat encounters various stress, such as dryness and low temperature, during cultivation. If plant growth regulators for tolerance toward stress can be used to improve yield, they will compensate for costly irrigation technologies and breeding for higher yields. In Japan, 'Norin No. 61' is widely cultivated in south of Kanto region. In 2010, cultivated acreage of 'Norin No. 61', which was registered as recommended variety by 15 of 47 prefectures, was 21983 hectare (Ministry of Agriculture, Forestry and Fisheries 2011). Although it is commonly cultivated in Japan, sensitivity to environmental stresses, particularly wet damage, has been reported. Wet damages at booting, heading, and ripening stages have often been reported. Wet conditions decrease yield of 'Norin No. 61' even though they produced enough number of ears (Hirano et al., 1964). The yield decrease is caused by decrease in number of grains per ear and grain weight (Yoshida et al., 1964). Yoshida et al (1964) have discussed that wet conditions inhibited fertilization or grain-filling. Oyanagi (2008) has found that plant height at the late growth stage correlates negatively with soil water content and positively grain yield, and pointed out importance to construct strategy for wet damages at the late growth stage. Wet damages have been known as not only fertilization and ripening damages, but also poor emergence and tillering (Oyanagi, 2008; Taya et al., 1981). 'Norin No. 61' was used in this study because it has been commonly cultivated in Japan, it has been sensitive to environmental stresses, and data about their responses towards environmental stresses have been accumulated. The relationships between its growth and meteorological factors have been studied in detail (Taya et al., 1981). Development of management technologies to overcome environmental stresses is clearly needed.

In this study in 2011 and 2012, we tried to verify that these compounds could contribute yield increase and to specify effective treatment periods for yield increase; in 2011, wheat was treated with AHX or ICA at seedling stage (we call it "seedling treatment") or after transplant of seedlings ("field treatment"). Since seedling treatment of the compounds increased wheat yield, wheat was treated with the compounds at earlier growth stage in 2012, namely the seeds were soaked in each compound's solution ("seed soaking treatment"). Here we describe the two-year's results.

2. Materials and Methods

2.1. Field examination in 2011

Field experiment was conducted at Center for Education and Research in Field Sciences, Shizuoka University (Fujieda city, 34º 54´N, 138º 16´E). Seeds of wheat cultivar 'Norin No.61' were sown in cell trays (one seed per cell, 8×16 cells, tray size 30×59×4.5 cm, cell volume 23.6 cm^2) with which culture soil (SB horticultural soil, Showa baido) was filled on 30 December 2010. Seeded trays were placed in the greenhouse with both sides open. When the third leaves of seedlings were expanded, they were transplanted to the field on 1 to 3 February 2011. Fertilizer was incorporated before transplant at N, P_2O_5, K_2O = 6.0, 7.2, 6.0 g m^{-2}. Row and plant spacing were 0.5 and 0.15 m respectively. Plant density was 13.3 m^{-2} (90 per plot). Rows were ridged by spade on 23 February 2011. There was no irrigation except for rainfall. Observation of morphological traits and harvesting were conducted on 14 June 2011. Harvested plants were dried in the greenhouse. Yield components were examined after threshing on 4 July 2011.

Wheat 'Norin No.61' was treated with AHX or ICA at seedling stage (we call it "seedling treatment") or after transplant of seedlings ("field treatment") to characterize effects of the two substances in field.

In seedling treatment, 1 L of 1 mM AHX or 0.1 mM ICA aqueous solution was poured over germinated seedlings of wheat in a cell tray by sprinkling can every day from 18 to 31

January, 2011. The treatments started at leaf age of 1.5 and ended at the age of 3. No tillers were developed during seedling treatment.

In field treatment, 6 L of 0.1, 0.5 mM AHX or 0.01, 0.05 mM ICA respectively were used to treat the plot (6.75 m^2) each time. They were poured to basal stems of the plant by sprinkling can. Treatments were performed 6 times, and the dates were 4, 18 February, 4, 18 March, and 1, 15 April. Plots of seedling treatment were irrigated with 6 L of water. The treatments started at leaf age of 3 and ended when the flag leafs started to expand.

A randomized plot design with triplicates was employed. The longest stem and ear length of a plant were measured, 15 plant per plot on 14 June. The wheat was harvested as 11 bundles of 5 in each treatment. After harvesting, number of ears, dry weight of whole plant, grain weight per plant and weight of 1000 grains were measured. Grain weight per plant and number of ears par plant were converted to per m^2 by multiplication each value per plant and plant density together. Dunnett's test was used to test significant differences between control and treatment.

Temperature and precipitation data were referred from the nearest climate monitoring system of Shizuoka Local Meteorological Observatory (34º 59´N, 138º 24´E; Shizuoka Local Meteorological Observatory, 2013).

2.2. Field examination in 2012

Germination of seeds was stimulated by soaking in water, or AHX or ICA solution (seed : water or solution = 1:3 parts in volume), shaking at 140 rpm by reciprocating shaker for 36 hours in dark at 25°C and air-dried for 12 hours. Germinated seeds were sown in field on 28 December 2011. Fertilizer was incorporated before sowing at N, P_2O_5, K_2O = 6.0, 6.0, 6.0 g m^{-2}. Row and plant spacing were 0.4 and 0.1 m respectively. Plant density was 25 m^{-2} (75 per plot). There was no irrigation except for rainfall. Number of tillers was observed on 27 February, 13, 28 March and 17 April 2012. Observation of morphological traits and harvesting were conducted on 20 June. Harvested plants were dried in the greenhouse. Yield components were examined after threshing on 4, 10 and 17 July.

Wheat was treated with AHX or ICA at seed soaking stage (we call it "seed soaking treatment") or by field treatment to characterize effects of the two substances in field.

In seed soaking treatment, seeds were soaked on 1 mM AHX or 0.1 mM ICA aqueous solution for 36 h at the time of stimulation of germination.

In field treatment, 5 L of 0.1, 0.5 mM AHX or 0.01, 0.05 mM ICA was used to treat a plot (3 m^2) each time. They were treated to basal stems by sprinkling can. Treatments were performed 4 times (twice before emergence of seedlings and twice after the start of elongation of lower internodes), and treatment dates were 28 December, 11 January, and 6, 20 April.

A randomized plot design with triplicates was employed. Controls, which were treated with water, were arranged in seed soaking treatments plots and field treatment plots respectively. Plant length and number of tillers were observed as growth characteristics. Their traits were measured, 10 plant per plot on 27 February, 13, 28 March and 17 April. Tillering did not start on 27 February. The longest stem, ear length and number of ears of a plant were measured, 10 plant per plot on 20 June. The wheat was harvested as 6 bundles of 5 in each treatment. After harvesting, dry weight of whole plant, grain weight per plant, weight of 1000 grains and grain weight per ear were measured.

Other than experimental procedure mentioned above, the same procedure as 2011 was employed.

3. Results

3.1. Field examination in 2011

Table 1 shows effects of AHX or ICA on yield, yield components and morphological traits of wheat 'Norin No.61' in 2011. By seedling treatments, 1.0 mM AHX increased yield (grain weight per m^2) up to 10.2 % significantly and 0.1 mM ICA showed tendency to increase up to 5.2 %. As to yield components, AHX increased number of ears up to 10.7 % significantly and ICA showed tendency to increase up to 5.3 %. There were no differences between treated plants and control in grain weight per 1000 seeds and per ear. As to morphological traits, AHX showed significant increase effects on culm length and dry weight up to 2.4 % and 9.7 % respectively.

By field treatments, 0.01 mM and 0.05 mM ICA showed significant increase effects on yield up to 8.8 % and 11.3 % respectively. 0.1mM AHX showed tendency to increase up to 7.8 %. As to yield components, 0.01 mM ICA showed tendency to increase number of ears up to 7.2 % and 0.05 mM significant increase up to 10.0 %. There were no differences between treated plants and control in grain weight per 1000 seeds and per ear, except for 0.1 mM AHX showing tendency to increase grain weight per ear up to 4.2 %. As to morphological traits, 0.1 mM AHX and 0.05 mM ICA increased dry weight up to 8.2 % significantly.

3.2. Field examination in 2012

Table 2 shows effects of AHX or ICA on yield, yield components and morphological traits of wheat 'Norin No.61' in 2012. By seed soaking treatments, 1.0 mM AHX increased yield up to 20.4 % significantly and 0.1 mM ICA showed tendency to increase up to 9.8 %. As to yield components, AHX increased number of ears up to 20.8 % significantly and ICA showed tendency to increase up to 12.4 %. There were no differences between treated plants and control in grain weight per 1000 seeds and per ear. As to morphological traits, AHX showed significant increase effects on ear length and dry weight up to 8.4 % and 18.3 % respectively.

By field treatments, 0.5 mM AHX and 0.05 mM ICA showed tendency to increase effects on yield up to 4.0 % and 10.1 % respectively. As to yield components, 0.05 mM ICA showed tendency to increase number of ears up to 6.0 %. There were no differences between treated plants and control in grain weight per 1000 seeds and per ear, except for 0.5 mM AHX showing tendency to increase grain weight per ear up to 5.3 %. As to morphological traits, 0.05 mM ICA showed tendency to increase dry weight up to 9.2 % significantly.

Fig.2 shows effects of AHX or ICA on number of tillers by seed soaking treatment method and field treatments through the cultivation in 2012. Number of tillers by seed soaking treatments increased drastically through the cultivation in AHX treatment (20.4% on 76 DAS, 19.5% on 91 and 17.1% on 111; Fig.2).

By field treatment, number of tillers on 76 DAS showed tendency to increase 14.2 % by 0.1 mM AHX. Those on 111 DAS showed tendency to increase 2.8% and 3.6% by 0.5 mM

AHX and 0.05 mM ICA, respectively.

4. Discussion

4.1.Effects of seed soaking treatment on wheat yield

In 2011, seedling treatment of AHX increased wheat yield significantly and ICA also showed the tendency to increase the yield by treatment (Table 1). The period of the seedling treatment was between leaf age of 1.5 and 3. This result indicated that the wheat was exposed to the compounds when it was at the early development just before tillering and encouraged to us to try next experiment at earlier growth stage than seedling, namely, treatment of wheat seeds with the compounds. As a result, AHX treatment increased yield significantly and treatment with ICA indicated the tendency to increase like seedling treatment (Table 2). These results indicate that both the compounds, at least AHX can increase wheat yield at early growth stage, even only at germination stage.

4.2. Effects of field treatment on wheat yield

In 2011, field treatment of ICA increased wheat yield significantly (Table 1). Field treatment started on 4 February (38 DAS) just after transplant and finished on 15 April (108 DAS) when flag leaf started to emerge. Li et al. (1993) has reported that intermodal elongation started on 126 DAS, and maximum number of tillers was attained on 125 to 130 DAS. Tillering is considered to finish until starting of internodal elongation (Li et al., 1993). In 2011, although 15 April was 108 DAS and in the middle of internodal elongation stage, it seemed that maximum number of tillers had already been attained on the day. Shanahan et al. (1985) have shown that tillering started on March (mean daily temperature is 0.8°C) when mean daily temperature changed from minus to plus, and the maximum number of tillers was attained in the middle of April (6.8°C). In 2011, mean daily temperatures in February and March were 8.8°C and 8.9°C respectively (Fig. 3). These observations and the literature data indicate that the peak of tillering was attained on March and the wheat was treated with the compound at tillering stage in the field experiment in 2011. From all the results and consideration, we hypothesized that treatment with the compound at early developmental stage and during internodal elongation close to the peak of tillering could contribute to increase yield, wheat was treated with the compound 4 times (on 28 December, 11 January, 6 April, and 20 April) in field treatment in 2012. As a result, 0.05 mM ICA treatment tended to increase yield (Table 2). The two-year results of field treatments indeed showed that ICA could increase wheat yield, but the timing of treatment of the compound needs to be examined under various conditions for more years.

4.3. Factors of yield increase: yield component

Whenever the yields of wheat were increased by treatments with AHX or ICA, the numbers of ears also increased (Tables 1 and 2). AHX and ICA did not affect grain weights per 1000 grains and per ear in any of the treatments. Grain weight per plant of rice cultivated in pot with AHX increased up to 25% (Control 36.9 g plant^{-1}, AHX 46.3) and number of ears per plant increased 11% (Control 27.3 plant^{-1}, AHX 30.8, Choi et al., 2010a). Those results indicated that yield increase was mainly caused by number of ears. Number of ears depends on multiplication between number of tillers and percentage of productive tillers. In 2012, there was no significant difference between control and the treated wheat (Table 3). These results indicate that both the compounds increased the number of ears by increasing the number of tillers.

It has been reported that number of grains per area and number of ears per area showed positive correlation with each other and yield of wheat depended on multiplication between number of ears and grain weight per ear (Fukushima et al., 2001; Ueda et al., 2000). These reports mean that increasing the number of ears is important for increase in yield. Narrow-sense heritability for number of tillers per plant is lower than that for number of grains per ear (Gill et al., 1972). It means that number of ears is easy to be affected by environmental conditions than number of grains per ear. Increasing the number of ears by AHX or ICA treatment could be the result of mitigation of some of environmental stress.

4.4. Factor of yield increase: relation with stress

Number of tillers tends to be affected by environmental conditions. It has been reported that increasing temperature, decreasing radiation (Thorne and wood, 1987) and non-irrigated conditions (Davidson and Chevalier, 1990) decreased number of tillers. Taya et al. (1981) analyzed results of ‘Norin No. 61’ cultivation examination throughout 28 years and reported that number of ears decreased in years of relatively high temperature between tillering stage and early stage of internodal elongation (December to March). Based on those results, accumulated temperature from December to March was from 640°C to 1050°C throughout 28 years (Taya et al., 1981). In 2011, accumulated temperature from February until April, corresponding to tillering stage and early stage of internodal elongation, was 963.3°C (Fig. 3). In 2012, accumulated temperature from March until April, corresponding to tillering stage and early stage of internodal elongation, was 767.2°C (Fig. 3). As compared with the report of Taya et al. (1981), the accumulated temperature in 2011 was a worse condition for increasing number of tillers. The results in this study shows that AHX or ICA treatment might nullify the effect of decrease in tillers under higher temperature during tillering stage.

4.5. AHX or ICA mechanisms for increasing wheat yield

Studies about symbiotic associations between grasses (*Lolium* and *Festuca* spp.) and *Neotyphodium* spp. endophytes that symbiotic fungi supply host plants with stress tolerance have been reported. Enhanced accumulation of osmotically active metabolites was suggested as inducing tolerance in drought-stressed endophyte-infected grasses (Elmi and West, 1995). None of the known fungal metabolites in symbiotic grasses appear to be directly involved in regulation of osmotic adjustment (Malinowski et al., 2005). Drought-stressed endophyte-infected grasses show an increased regrowth rate (Bacon, 1993). One of possible factors is indole acetic acid (IAA) produced by endophytes (Bacon, 1993). At the other extreme, endophyte-infected grasses under waterlogged conditions produced rhizome above the water surface and more dry matter than uninfected ramet (Bacon, 1993). It is possible that bioactive compounds which can regulate plant growth suffered exactly opposite stresses, like drought and waterlogged stresses, exist in unknown symbiotically fungal metabolites.

In the earlier paper, it was reported that rice seedlings treated by AHX showed that expression of glutathione *S*-transferases (GST) and Bowman-Birk-type proteinase inhibitor (BBI) genes were up-regulated 3 to 9 times, and AHX treatment increased rice yield (Choi et al., 2010a). It has also been known that both these genes are closely related to tolerance due to environmental stress like sodium chloride and low temperature (Shan et al., 2008; Zhao and Zhang, 2006), therefore, we concluded that the increase in rice yield was due to AHX-induced tolerance to environmental stress (Choi et al., 2010a). The increase of the number of ears in this study also could be explained due to tolerance induced by AHX or ICA treatment. It is necessary for future work to understand AHX or ICA mechanisms for mitigating to environmental stress and relationship between stress tolerance and yield increase of wheat.

As stated above, we proved the existence of endogenous AHX in various plants such as rice, tomato, potato, *Arabidopsis* and so on (Choi et al., 2014). In addition, we just confirmed that wheat also contains AHX (data not shown). We believe that ICA also exists in plants endogenously but the method of detection of ICA has not been established yet. Wheat itself may control its growth by using these compounds.

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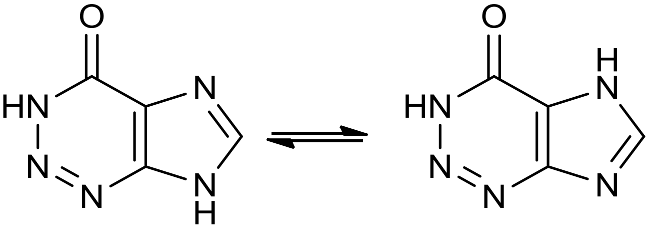
Captions

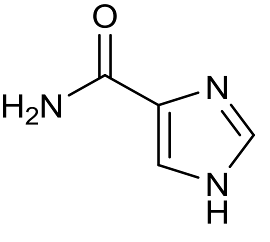
Fig. 1. Structure of 2-azahypoxanthine (AHX) and imidazole-4-carboxamide (ICA).

Fig. 2. Effects of AHX or ICA on number of tillers (m^{-2}) of wheat 'Norin No.61' in 2012. * indicates significant difference from control.

Fig. 3. Summary of monthly mean daily temperatures, accumulated temperature and total precipitation during growing seasons at Shizuoka City (Shizuoka Local Meteorological Observatory, 2013).



AHX



ICA

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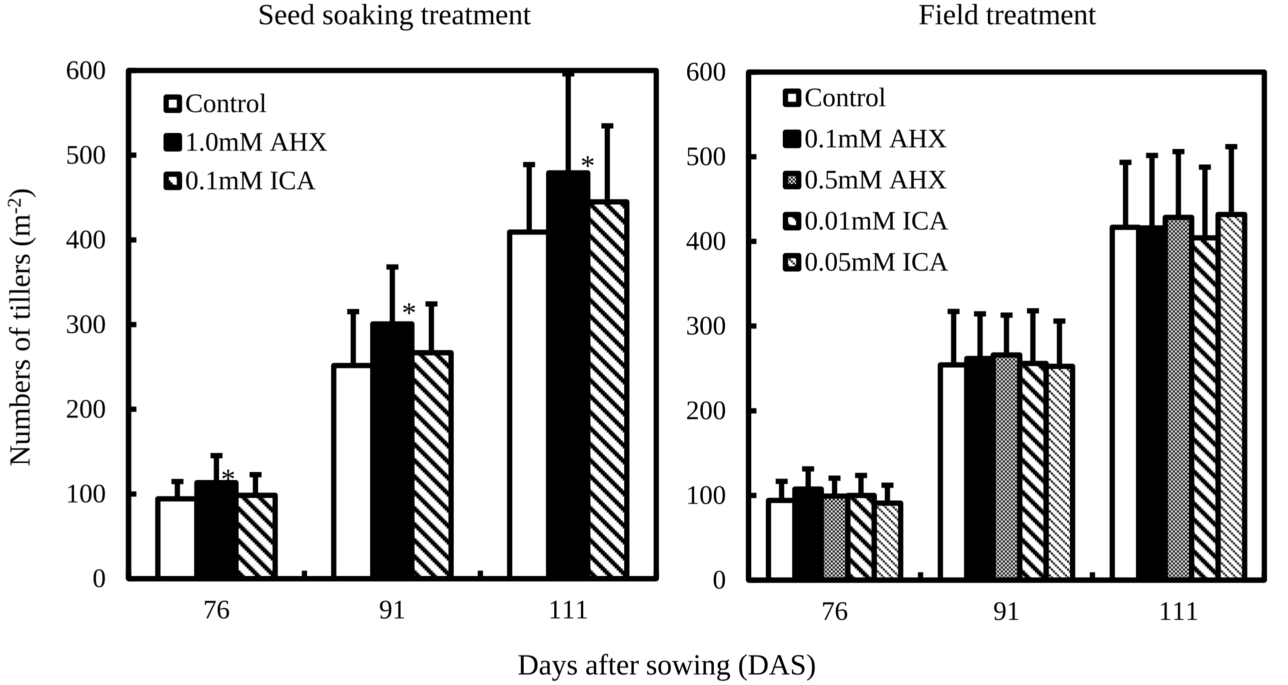

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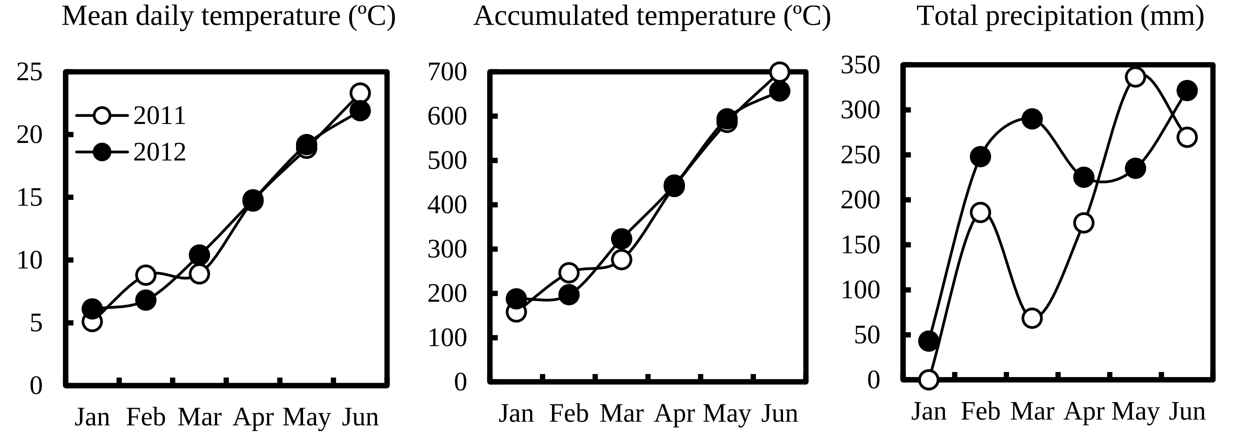

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Table 1

Effects of AHX or ICA on yield, yield components and morphological traits of wheat 'Norin No.61' in 2011.

Trait	Control	Seedling treatments			
		AHX		ICA	
		Concentration (mM)			
		1.0		0.1	
Yield					
Grain weight (g m^{-2})	336.6 ± 43.4	371.1 ± 46.6*	(10.2 %)	355.5 ± 40.8	(5.6 %)
Yield components					
Number of ears (m^{-2})	217.0 ± 23.3	240.2 ± 27.6*	(10.7 %)	228.4 ± 26.5	(5.3 %)
Grain weight (g 1000 $grains^{-1}$)	38.3 ± 2.0	36.9 ± 0.8	(-3.7 %)	36.6 ± 1.4	(-4.5 %)
Grain weight (g ear^{-1})	1.55	1.54	(-0.4%)	1.56	(0.3%)
Morphological traits					
Culm length (cm)	79.8 ± 4.7	81.7 ± 3.1*	(2.4 %)	80.6 ± 3.4	(1.0 %)
Ear length (cm)	9.0 ± 0.5	9.2 ± 0.6	(2.5 %)	8.8 ± 0.7	(-2.0 %)
Dry weight (g $plant^{-1}$)	55.6 ± 7.5	61.0 ± 7.1*	(9.7 %)	57.9 ± 6.5	(4.0 %)

Trait	Control	Field treatments							
		AHX				ICA			
		Concentration (mM)							
		0.1		0.5		0.01		0.05	
Yield									
Grain weight (g m^{-2})	336.6 ± 43.4	363.0 ± 43.6	(7.8 %)	344.4 ± 46.4	(2.3 %)	366.2 ± 55.4*	(8.8 %)	374.6 ± 43.2*	(11.3 %)
Yield components									
Number of ears (m^{-2})	217.0 ± 23.3	224.6 ± 18.3	(3.5 %)	219.0 ± 27.8	(0.9 %)	232.5 ± 25.9	(7.2 %)	238.6 ± 25.4*	(10.0 %)
Grain weight (g 1000 $grains^{-1}$)	38.3 ± 2.0	37.2 ± 0.7	(-2.8 %)	39.0 ± 2.4	(1.9 %)	36.7 ± 2.2	(-4.1 %)	37.4 ± 0.9	(-2.4 %)
Grain weight (g ear^{-1})	1.55	1.62	(4.2%)	1.57	(1.4%)	1.57	(1.5%)	1.57	(1.2%)
Morphological traits									
Culm length (cm)	79.8 ± 4.7	80.1 ± 3.5	(0.4 %)	80.2 ± 2.9	(0.5 %)	80.2 ± 2.8	(0.5 %)	81.6 ± 3.2	(2.3 %)
Ear length (cm)	9.0 ± 0.5	9.1 ± 0.7	(1.0 %)	9.1 ± 0.6	(0.5 %)	8.9 ± 0.8	(-1.5 %)	8.9 ± 0.7	(-0.9 %)
Dry weight (g $plant^{-1}$)	55.6 ± 7.5	60.2 ± 6.7*	(8.2 %)	56.1 ± 7.7	(0.9 %)	59.4 ± 7.8	(6.8 %)	60.2 ± 6.9*	(8.2 %)

Mean ± Standard Deviation. Increase or decrease rate for control in parentheses. * Significant difference at $P < 0.05$ from control (Dunnett's test).

Table 2

Effects of AHX or ICA on yield, yield components and morphological traits of wheat 'Norin No.61' in 2012.

Trait	Control	Seed soaking treatments			
		AHX		ICA	
		Concentration (mM)			
		1.0		0.1	
Yield					
Grain weight (g m^{-2})	500.6 ± 100.2	602.5 ± 120.8*	(20.4 %)	549.4 ± 95.9	(9.8 %)
Yield components					
Number of ears (m^{-2})	375.8 ± 73.0	454.2 ± 116.5*	(20.8 %)	422.5 ± 90.8	(12.4 %)
Grain weight (g 1000 $grains^{-1}$)	36.4 ± 2.5	36.5 ± 3.0	(0.4 %)	35.1 ± 2.8	(-3.4 %)
Grain weight (g ear^{-1})	1.33	1.33	(-0.4%)	1.30	(-2.4 %)
Morphological traits					
Culm length (cm)	76.1 ± 3.0	77.6 ± 3.2	(1.9 %)	78.1 ± 4.2	(2.6 %)
Ear length (cm)	8.5 ± 0.6	9.2 ± 0.7*	(8.4 %)	8.6 ± 0.8	(1.8 %)
Dry weight (g $plant^{-1}$)	41.0 ± 6.8	48.5 ± 8.5*	(18.3 %)	45.1 ± 6.9	(10.1 %)

Trait	Control	Field treatments							
		AHX				ICA			
		Concentration (mM)							
		0.1		0.5		0.01		0.05	
Yield									
Grain weight (g m^{-2})	483.9 ± 76.9	459.4 ± 139.9	(-5.1 %)	503.1 ± 99.9	(4.0 %)	471.4 ± 79.5	(-2.6 %)	532.8 ± 130.7	(10.1 %)
Yield components									
Number of ears (m^{-2})	386.7 ± 92.6	387.5 ± 94.2	(0.2 %)	381.7 ± 89.5	(-1.3 %)	368.3 ± 65.0	(-4.7 %)	410.0 ± 105.8	(6.0 %)
Grain weight (g 1000 $grains^{-1}$)	35.7 ± 3.5	35.0 ± 3.6	(-2.1 %)	36.4 ± 2.2	(1.9 %)	37.8 ± 1.5	(5.9 %)	36.1 ± 3.5	(1.1 %)
Grain weight (g ear^{-1})	1.25	1.19	(-5.3%)	1.32	(5.3 %)	1.28	(2.3 %)	1.30	(3.8 %)
Morphological traits									
Culm length (cm)	75.3 ± 3.8	75.6 ± 4.3	(0.4 %)	74.3 ± 4.8	(-1.3 %)	73.7 ± 2.6	(-2.1 %)	74.9 ± 4.1	(-0.5 %)
Ear length (cm)	8.6 ± 0.8	8.5 ± 0.7	(-0.2 %)	8.6 ± 0.5	(1.0 %)	8.4 ± 0.6	(-1.9 %)	8.7 ± 0.9	(1.6 %)
Dry weight (g $plant^{-1}$)	39.0 ± 5.7	38.8 ± 9.5	(-0.5 %)	40.5 ± 7.5	(3.7 %)	37.0 ± 6.6	(-5.3 %)	42.6 ± 9.5	(9.2 %)

Mean ± Standard Deviation. Increase or decrease rate for control in parentheses. * Significant difference at $P < 0.05$ from control (Dunnett's test).

Table 3

Effects of treatments of AHX or ICA on the percentage of productive tillers[a] of wheat 'Norin No.61' in 2012.

1. Seed soaking treatment

Control	AHX	ICA
	Concentration (mM)	
	1.0	0.1
91.9	94.8	94.9

2. Field treatment

Control	AHX		ICA	
	Concentration (mM)			
	0.1	0.5	0.01	0.05
92.8	93.2	89.1	91.1	95.0

[a] The percentage of number of ears to number of tillers on 17 April.