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Improvement of Chilling Resistance in Rice by Application of an Abscisic Acid Analog in Combination with the Growth Retardant Tetcyclacis

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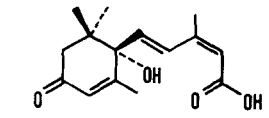
Abstract. The protective effect of a synthetic terpenoid analog of the plant hormone abscisic acid (ABA) coded LAB 173711 applied alone or in combination with the growth retardant tetcyclacis on chilling injury of rice was studied under growth chamber, greenhouse, and field conditions. The compounds were applied as a foliar spray before and after the onset of chilling treatment, as a substrate via the root system, and as a medium for seed soaking. The ABA analog increased chilling resistance in a manner similar to ABA. Combination of the analog with tetcyclacis revealed additive effects. Increased chilling resistance involved several processes: stomatal closure which reduced water loss during chilling, stabilization of the membranes, stabilization of the chlorophyll level, and stabilization of the root system. Possibilities for practical use of the compounds in rice production are discussed.

Rice cultivation in temperate and high-altitude areas is limited by rice susceptibility to low air temperature and irrigation water temperature. An estimated 7 million ha of rice is grown in cold areas in South and Southeast Asia. Developing suitable cold-tolerant varieties and improved cultural practices offer immense possibilities for increasing rice yield in such areas. Some progress has been achieved with the introduction of cold-tolerant *Japonica* cultivars in breeding programs. Further progress is seen from the use of short-duration varieties that avoid, rather than tolerate, cold injury. Also, cold damage can be avoided through cultural practices such as the use of plastic shelters to protect seedbeds. Attempts to protect rice seedlings or plants against low temperature using plant growth regulators have also been reported.

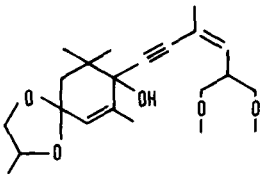
The plant hormone abscisic acid (ABA) is involved in several stress reactions of plants including low temperature stress (Capell and Dörffling 1989). It is, therefore, not surprising that ABA application has a protective function against chilling, freezing, and drought stress. Rikin et al. (1976, 1979, 1983) were the first to describe that spraying ABA on cucumber seedlings prior to a chilling stress increased chilling tolerance. Flores et al. (1988) and Flores and Dörffling (1990) extended the number of plant species that responded to ABA application with an increase in chilling resistance. Among these plants was rice (Flores-Nimedez et al. 1990a,b). In spite of its high activity, the use of ABA on a large scale is limited because of its high price and its rapid metabolic deactivation. Several attempts to introduce synthetic ABA analogs as protectants against chilling and freezing damage in horticulture and agriculture have failed in the past mainly because of the low activity of the compounds used.

Recently, new ABA analogs have been synthesized (Grossman and Jung 1984). In these compounds, the dienoic side chain of ABA was replaced by a conjugated alkene–alkine unit, the carboxyl group substituted by an acetal or ether function, and the 4′-keto group in the cyclohexenyl ring replaced by hydrogen or by a cyclic ketal (Fig. 1). Although these compounds are available so far only as impure preparations with several isomers, their biological activity in various physiological processes (inhibition of seed germination, promotion of abscission, induction of stomatal closure) is qualitatively and quantitatively similar to that of the natural hormone (Flores and Dörffling 1990). They seem to be more stable in the plant and their chemical synthesis is easier and cheaper.

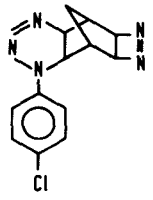
The present study aims to demonstrate the ability of one of these analogs to increase chilling tolerance and prevent cold damage in rice. It also reports ex-



Abscisic acid
ABA



LAB 173711



Tetcyclacis
Tet

Fig. 1. Chemical structure of abscisic acid (ABA), its terpenoid analog LAB 173711, and tetcyclacis, used as protectants against chilling injury in rice.

periments which show that combinations of the new ABA analog with another synthetic plant growth regulator, tetcyclacis, a norbornadiazetine-type plant growth retardant (Fig. 1), have additive effects on chilling protection. Recently, it has been reported that tetcyclacis, known to block a distinct step in gibberellin biosynthesis (Rademacher et al. 1987), also improves cold tolerance (Anderson and Husband 1987). Also included are studies on the mode of action of these compounds in preventing chilling damage and discussions on the possibilities and limits of their application in rice production.

Materials and Methods

Preparation of the Test Solutions

Preparations of the analog LAB 173711 (Fig. 1) (provided by Dr. W. Rademacher, BASF Agricultural Research Center, Limburgerhof, Germany) were dissolved in cyclohexanone or acetone (Merck, Darmstadt, Germany). The solutions were diluted with distilled water containing 0.025% Citowett (BASF) as additive wetting agent to obtain the following growth regulator concentrations: 10^{-4} and 10^{-3} mol L^{-1}. The concentration of the solvent was 1% or less. ABA, a racemic mixture of the (+)- and the (−)-isomer (Fluka, Basel, Switzerland), and tetcyclacis (provided by Dr. W. Rademacher, BASF) were prepared in the same manner.

Plant Material

Seeds of *Oryza sativa* L. cv IR 36 and Samgangbyeo (chilling-sensitive cultivars) were surfaced-sterilized with 0.1% mercuric chloride solution for 1 min and washed with distilled water. Seeds were soaked in distilled water for 24 h, lined in petri dishes with moistened filter paper, and incubated overnight at 32°C. The germinated seeds were transferred to 1-L pots of Maahas clay soil at 29°/21°C in the glasshouse or greenhouse under natural photoperiod, or in the growth chamber at 26°/21°C with a 12-h photoperiod and 80% relative humidity.

Application Techniques and Chilling Treatments

Different application methods and chilling treatments were used.

Foliar Spray. Two- and 3-week-old IR36 seedlings were sprayed with 10^{-4} and 10^{-3} mol L^{-1} LAB 173711, respectively, until the leaves were completely wet. After 24 h, the 3-week-old plants were transferred to the cold air (5°C and 11°C) treatment for several days. The 2-week-old plants were transferred to cold irrigation water (water tank) with a temperature of 11°C for 7 days, the shoots exposed to 29°C. At the end of the chilling exposure, the 2-week-old seedlings were transplanted to the field.

Flower Spray. A set of IR36 plants were raised in the greenhouse at 32°C. Before anthesis, intact plants with flowers were sprayed with 10^{-4} mol L^{-1} ABA and LAB 173711. After 2 days, plants were transferred to 11°C for 9 days and returned to 32°C for recovery. After 134 days from seed sowing, panicles of the main tiller from five different plants per treatment were harvested, percent of filled spikelets and grain weight were monitored.

Another set of plants, cv Samgangbyeo, were grown in the glasshouse at 29°/21°C. Before anthesis, plants were sprayed with 10^{-4} mol L^{-1} ABA, LAB 173711, and the combination of LAB 173711 + tetcyclacis. After 3 days, plants were transferred to 5°C for 7 days and then returned to 29°C for recovery until the harvesting or full ripe period (127 days after seed sowing). The panicles of the main tillers were harvested. Grain-filling presented in terms of filled spikelets and grain weight was measured.

Root Treatment. Two-week-old seedlings were transferred to a water cooled tank providing a root temperature of 11°C and shoot temperature of 29°C for 7 days. Afterwards, the roots were carefully removed from the soil, washed, then immersed in an aqueous solution of 10^{-4} mol L^{-1} LAB 173711 for 24 h and the seedlings were then transplanted to the field.

Seed Soaking. Seeds were soaked in distilled water for 24 h. Pregerminated seeds were soaked in an aqueous solution of 10^{-4} mol L^{-1} LAB 173711 for an additional 24 h, then sown in trays and raised in the greenhouse. Two-week-old seedlings were transferred to a cooled water tank providing a root temperature of 11°C and shoot temperature of 29°C for 7 days. The roots were pulled from the soil and seedlings transplanted to the field.

Measurement of Chilling Injury

Percent Survival. After exposure to chilling, a set of plants was transferred to the preexperimental condition. The number of surviving plants was counted.

Leaf Rolling. A visual scoring of rolling of the fifth leaf from the plant base was monitored by counting the number of completely rolled leaves before, during, and after chilling at 5°C, using a scale of 1 to 5.

Changes in Relative Chlorophyll Content. The mid-lamina of the third leaf from the top was used to measure relative chlorophyll content using SPAD-501 (Minolta Camera Co., Ltd., Osaka, Kokusai, Japan).

Chlorophyll Fluorescence Measurements. Twenty-seven-day-old IR36 rice seedlings were grown in the glasshouse at 29°C, chilled at 5°C for 3 days, and then transferred to a dark room for a 2-h preincubation period. The seventh intact leaf of the plant rested on a flat surface. The photodiode, which detects the fluorescence light was placed on the adaxial surface of the mid-leaf lamina, and leaf chlorophyll fluorescence was measured with a PAM chlorophyll fluorometer (Heinz Walz Mess- u. Regeltechnik, FRG). The fluorescence of the leaf sample was excited at frequencies of 1.6 or 100 KHz by 1 us light pulses from a light-emitting diode (LED). The LED-measuring beam has a peak wavelength of 650 nm. The signal from the photosensor was registered on a recorder. Variable fluorescence (F_v, where $F_v = F_m - F_0$) was calculated from the measured instantaneous fluorescence (F_0) and the maximum fluorescence (F_m).

Results

Protection Against Cold Air Injury During the Seedling Stage

The effects of drastic low temperature treatment on IR36 seedlings and the protective function of the plant growth regulators are presented in Fig. 2. Three-week-old IR36 seedlings grown at 29°C temperature were sprayed with the respective solutions in a concentration of 10^{-3} mol L^{-1}. After 24 h, they were exposed to cold air treatment for 4 days, and thereafter returned to the original conditions. Percentages of plant survival, leaf rolling, leaf fresh weight, and relative chlorophyll content were recorded before, during, and after the low temperature treatment. Application of ABA, LAB 173711, tetcyclacis, and LAB 173711 + tetcyclacis before chilling exposure increased the capacity of the rice plants for survival. While untreated control plants did not survive after 10 days, all plants treated with ABA survived (see also Fig. 3). The survival percentage of plants treated with LAB 173711 was about 90% and of those sprayed with the combination of LAB 173711 and tetcyclacis was about 94%. Tetcyclacis alone was less active, but still showed a pronounced positive effect on plant survival. Parallel to the increase in seedling survival with the application of protective compounds, a significant reduction in the symptoms of chilling injury was observed. Leaf rolling was greatly reduced (about 60%) and reduction of leaf fresh weight and chlorophyll breakdown was prevented to about the same extent. The decrease in leaf water potential was minimized by about 40% (data not shown). Necrotic leaf area was about 40% in the control and less than 5% in LAB 173711-treated plants (data not shown). The decline in chlorophyll fluorescence was likewise minimized with only about a 20% decrease compared to about 80% in the control (Fig. 4). Not only were water status and chlorophyll contents affected, but membrane stability as well. A rapid increase in electrolyte leakage was observed during prolonged exposure of plants to low temperature. Application of LAB 173711 and the combination of LAB 173711 + tetcyclacis minimized this increase (data not shown).

Protection Against Cold Irrigation Water Injury During Seedling Stage and Comparison of Different Application Methods of the Protective Compounds

Chilling injury of rice plants may result not only from cold air, but also from cold irrigation water. In this experiment, the roots were exposed to a temperature of 11°C for 7 days, while the shots remained at the original temperature of 29°C. Thereafter the seedlings were transplanted to the field and percentage survival was monitored. Three different methods of cryoprotectant application were used and their effectivity compared. Of the three methods of application, foliar spray and root dipping proved to be more effective in preventing chilling injury, attaining 70% and 68% seedling survival, respectively (Fig. 5). Seed soaking was less effective compared to foliar spray and root dipping with only about 53% survival. It should be noted, however,

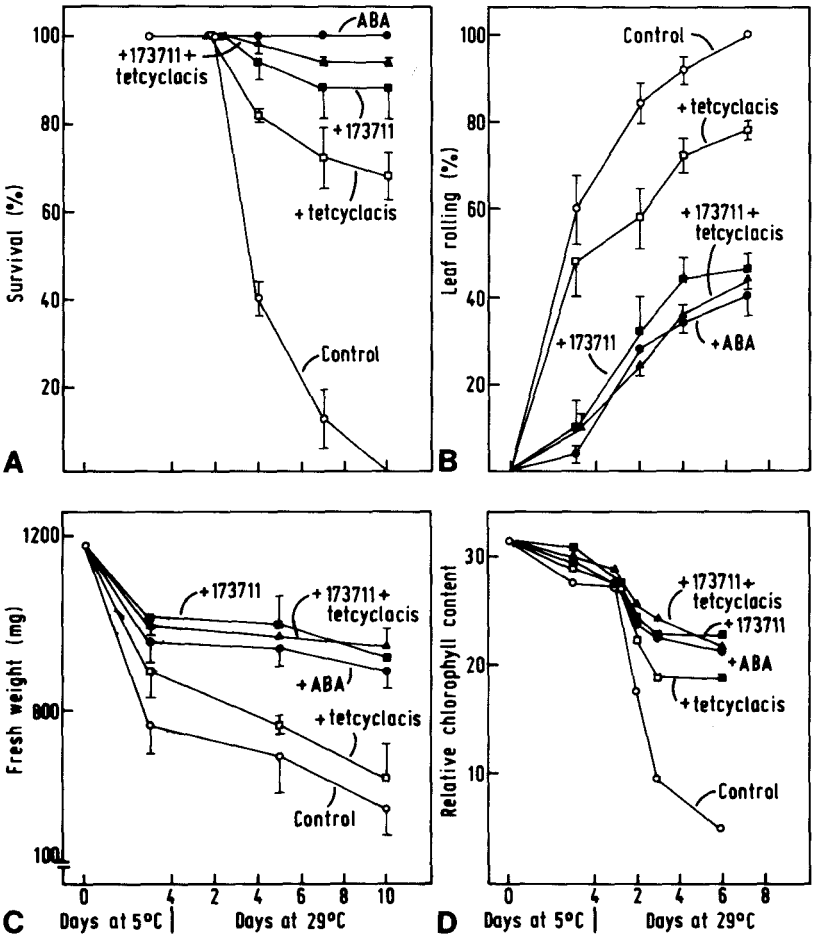


Fig. 2. (A) Percent survival, (B) inhibition of leaf rolling, (C) changes in leaf fresh weight, and (D) relative chlorophyll content of IR36 rice seedlings treated with a foliar spray of 10^{-3} mol L^{-1} ABA, LAB 173711, and tetcyclacis after a 24-h period of chilling at 5°C for 3 days. After the chilling treatment, the seedlings were retransferred to 29°C for recovery. Data are means ± SE of five parallel experiments with 10 plants each. The difference between control and treatment with tetcyclacis + LAB 173711 is significant at $\alpha = 0.01$ (Duncan's multiple range test).

that the time interval between analog application and the onset of chilling lasted 2 weeks with seed soaking. Part of the applied analog may have been metabolized during that period.

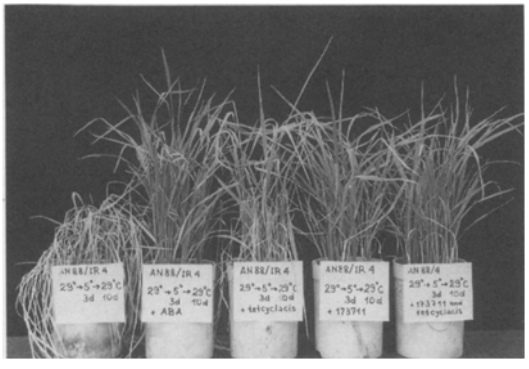


Fig. 3. No untreated rice plant (IR36) survived a chilling treatment (3 days at 5°C) after 10 days of recovery at 29°C. However, plants treated with a foliar spray of ABA, LAB 173711, and tetcyclacis showed a high percentage of survival.

Foliar application of the compounds and the subsequent exposure of the root system to cold water prevented the development of chilling symptoms in the shoot and also protected the roots. Reductions in root growth and root dry weight formation were significantly prevented not only by foliar application of LAB 173711 (Table 1) but also by using the protective compounds as root substrate (data not shown).

Protection Against Low Temperature Stress During the Flowering Stage

Since rice is susceptible to low temperature not only during the seedling stage but also during anthesis, the protective effects of the chemicals against low air temperature was also studied during the flowering stage. Figures 6 and 7 show an experiment in which seedlings of the chilling-sensitive Korean variety Samgangbyeo were grown in a glasshouse at temperatures of 29°/21°C. Before an-

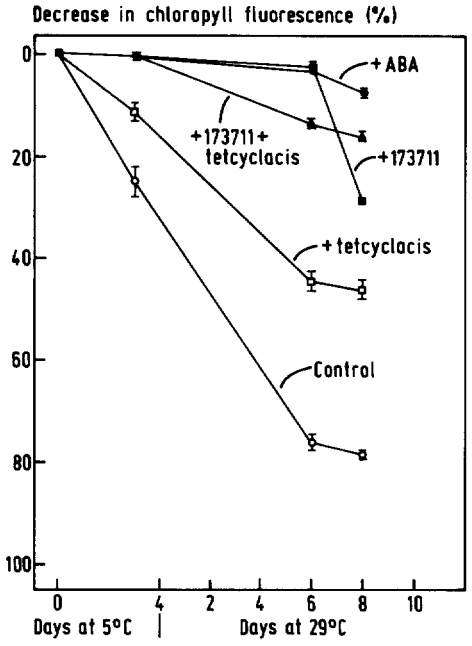


Fig. 4. Changes in chlorophyll fluorescence of rice seedlings (IR36) chilled at 5°C for 3 days, then retransferred to 29°C for recovery. Treatment with ABA, LAB 173711, tetcyclacis, and LAB 173711 + tetcyclacis at time zero as a foliar spray is shown. Data are means ± SE of 10 measurements. The difference between control and all treatments is significant at $\alpha = 0.01$ (Duncan's multiple range test).

thesis, the plants were sprayed with 10^{-4} mol L^{-1} ABA, LAB 173711, and the combination of LAB 173711 + tetcyclacis. Three days later, the plants were exposed to an air temperature of 5°C for 7 days and returned to a temperature of 29°C for recovery. The main panicles were harvested during the ripening stage (127 days after sowing). The yield and yield component analyses showed that chilling greatly reduced the percentage of filled spikelets (Fig. 6) and grain dry weight (Fig. 7). Application of ABA, LAB 173711, and LAB 173711 + tetcyclacis increased the percent of filled spikelets when sprayed before anthesis prior to the chilling treatment. The control, however, had a lower percentage of filled spikelets in both the primary and secondary branches. Most effective was the combination of LAB 173711 + tetcyclacis. Exposure to 5°C likewise caused a decrease in dry matter accumulation. The decrease was, however, smaller in those treated with ABA, LAB 173711, and the combination of LAB 173711 + tetcyclacis. The grain weight in both the upper and lower primary and secondary branches of the panicle was heavier in LAB 173711 + tetcyclacis-treated plants, but the differences to the chilled controls were statistically significant only in a few cases.

Discussion

Treatment with the synthetic ABA analog LAB 173711 can protect rice plants in different developmental stages against chilling stress. Combinations of the ABA analog with the growth retardant tetcyclacis, which by itself has also protective properties with respect to low temperature stress, have additive, sometimes even synergistic effects.

The transfer of chilling-sensitive plants from 29°C to 5°C may result in partial stomatal opening as reflected in terms of increased leaf rolling and fresh weight loss. Application of the ABA analog prior to chilling induces stomatal closure (Flores-Nimedez et al. 1990a) and, thus, protects the plant against injury. The water-conserving effect of applied ABA analog seems, moreover, to be the basis for its remarkable effect in preventing the "transplanting shock" of young rice seedlings during transfer from the seedbed to the field (Flores-Nimedez et al., in preparation).

Besides its direct effect on water status, the ABA analog protects the membrane system against chilling-induced inactivation and degradation. Membrane permeability as measured by electrolyte efflux is greatly stabilized by the application of ABA and its analog before chilling (Flores-Nimedez et al. 1990b).

Normally, foliar spray is performed 24 h or more before a chilling treatment. However, foliar spray and root dipping were also effective, although to a lesser degree, when applied immediately after the chilling treatment. This indicates that part of the ABA analog mode of action involves protection against chilling injury during the recovery period at higher temperatures rather than protection against the direct effects of low temperature.

Relative chlorophyll content and chlorophyll fluorescence were also used as criteria to measure injuries caused by chilling. The loss of chlorophyll may be due to the photooxidative damage to the photosynthetic apparatus (Hodgson and Raison 1984), thus a decrease in the chlorophyll content. Exposure of plants to chilling caused a fast decrease in chlorophyll fluorescence. This is in agreement with the findings of Greaves and Wilson (1986). Decreased fluorescence suggests that the oxidative site of photosystem II is the site of injury (Peeler and Naylor 1988, Smillie and Hetherington 1983). Analysis of chlorophyll fluorescence in rice by Moll and Steinback (1986) shows that low temperatures cause an increased reduction of the plastoquinone pool which could result in photoinhibitory damage to the photosystem II reaction centers. This decrease in fluorescence, however, is counteracted by the application of the ABA analog and tetcyclacis.

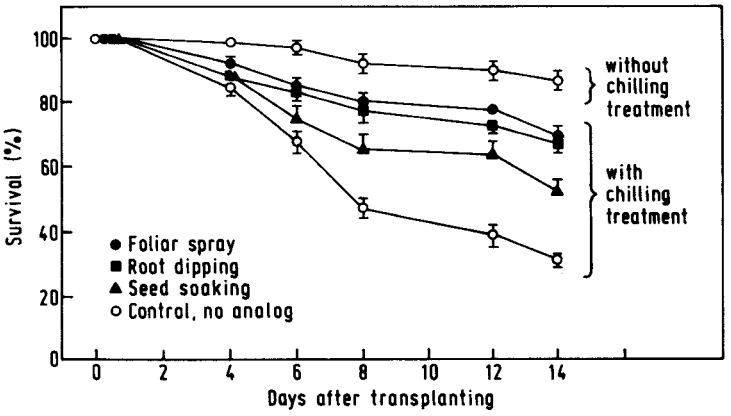


Fig. 5. Effect of 10^{-4} mol L^{-1} LAB 173711 applied with different methods on survival of IR36 seedlings—the roots of which were exposed to 11°C for 7 days. Afterward, the seedlings were transplanted to the field. Data are means ± SE of 24 measurements. The difference between control and all treatments is significant at $\alpha = 0.01$ (Duncan's multiple range test).

Table 1. Root growth of rice plants (IR36) treated with LAB 173711, chilled at 5°C for 2 days, and retransferred to 29°C for a 7-day recovery.

	Root growth			
	Nodal roots[a] (no./plant)	Length[b] (cm)	Fresh weight[b] (mg)	Dry weight[b] (mg)
Control	17.0 ± 1.8	9.9 ± 1.2	205.8 ± 28.3	27.7 ± 7.0
LAB #173711	26.2 ± 1.7	14.7 ± 2.2	770.8 ± 28.0	69.2 ± 7.0

[a] Data are means ± SE of five measurements.
[b] Data are means ± SE of eight measurements. The differences between treatment and control are significant at $\alpha = 0.01$ (Duncan's multiple range test).

The ABA analog can be taken up through the root system. Application of the ABA analog prior to chilling provided a protection of root growth. The stabilization of the root system may contribute to the increased survival rate in protected plants.

The mechanism of action of the synthetic ABA analog in preventing chilling damage is qualitatively identical with that of ABA. However, a direct quantitative comparison with ABA on a molar basis is not possible because preparations containing the analog are impure and contain several isomers whose biological activity is unknown. The ABA analog is active in the rice plant for a long time, as indicated by the observation that treatment of the seed may protect the seedling against chilling several weeks later. The analog seems, therefore, to be rather resistant to metabolic deactivation.

In all probability the synthetic analog acts by simulating the natural hormone. Enhanced levels of ABA after treatment with LAB 173711 have not been observed (Dörffling, unpublished observations). This mode of action seems to be different from the mode of action of tetcyclacis. Besides its inhibitory effect on gibberellin biosynthesis, tetcyclacis seems to act, at least partially, by inhibiting the transformation of endogenous ABA to its metabolites phaseic acid and dihydrophaseic acid thus increasing the level of ABA (Dörffling, unpublished results). Two other compounds with chilling-protective activity, triadimefon and mefluidide, also seem to exert their action, at least in part, by increasing the endogenous level of ABA (Asare-Boamah and Fletcher 1986, Zhang et al. 1986). The direct action of LAB 173711 may be advantageous over the indirect action of mefluidide and triadimefon in regard to their practical applicability.

Since cold tolerance in rice can be increased by applying ABA, its analog, or compounds that increase the endogenous level of ABA, selecting and breeding for "high-ABA plants" may be a promising way to increase cold tolerance in rice. No information is available whether this has been tried. With drought tolerance improvement, however, few attempts have been reported. Quarrie (1987) succeeded with the classical selection and crossing methods in producing wheat plants with increased capacity to produce ABA in response to drought and improved water use efficiency.

Since there are no rice cultivars with sufficient cold tolerance, cryoprotective chemicals may be useful in preventing chilling damage and thus stabilizing yield. The compounds reported here have the advantage of high activity, especially when used in combinations. However, their high price is a disad-

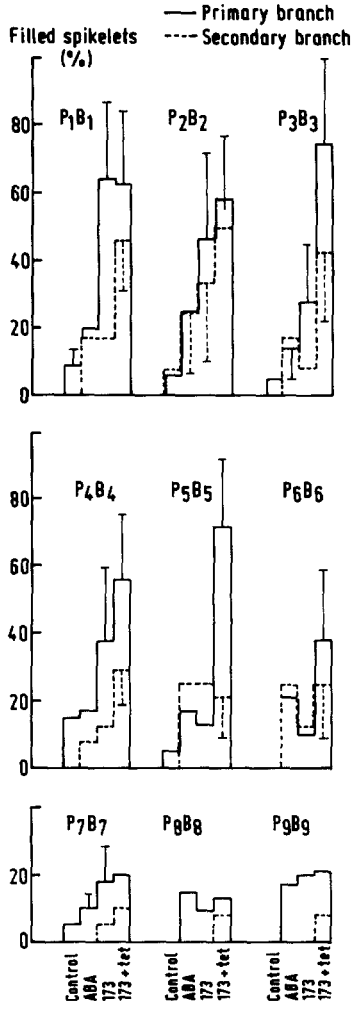


Fig. 6. Effect of 10^{-4} mol L^{-1} ABA, LAB 173711, and LAB 173711 + tetcyclacis on the percent of filled spikelets in the variety Samgangbyeo. Before anthesis, the plants were sprayed with the compounds. After 3 days they were exposed to 5°C for 7 days and then returned to 29°C. The main panicles were harvested during the full ripe period. Mean ± SE of four parallel experiments. Control, chilled plants not treated with the protective compounds; ABA, abscisic acid; 173, LAB 173711; 173 + tet, combination of LAB 173711 + tetcyclacis. P_1B_1, P_2B_2, P_3B_3 . . . PgBg, 1st, 2nd, 3rd . . . 9th primary rachis branch, respectively; location in the rachis of the panicle from where the spikelets were obtained. For example: P_1B_1, spikelets were collected from the 1st primary rachis branch.

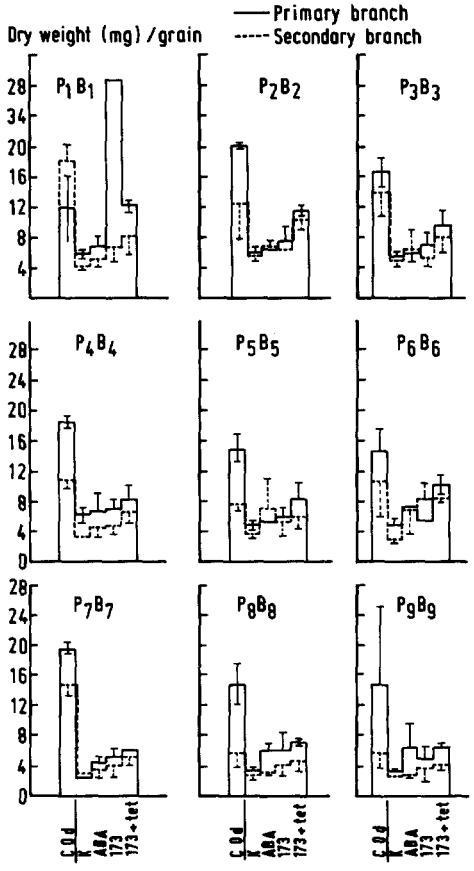


Fig. 7. Effect of 10^{-4} mol L^{-1} ABA, LAB 173711, and LAB 173711 + tetcyclacis on grain dry weight in the variety Samgangbyeo. Before anthesis, the plants were sprayed with the compounds. After 3 days they were exposed to 5°C for 7 days and then returned to 29°C. The main panicles were harvested during the full ripe period. The same experiment as in Fig. 6. C, control; unchilled plants, not treated with the protective compounds; K, chilled plants not treated with the protective substances; ABA, abscisic acid; 173, LAB 173711; 173 + tet, combination of LAB 173711 + tetcyclacis. See Fig. 6 for abbreviations.

vantage for large-scale application in agriculture. Further studies seem necessary to determine if small-scale application would be economically feasible (e.g., when seedlings from plastic shelters are transferred to the field under low temperature conditions). Also, promising results have been reported on the synthesis of ABA analogs of even higher activity and easier production (Schubert et al. 1991).

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References

Anderson HM, Huband NDS (1987) Improvement of winter hardiness and seedling growth of oats with seed dressings of tetcyclacis. In: Hawkins AF, Stead AD, Pinfield NJ (eds) Plant growth regulators for agricultural and amenity use. Monograph No. 36, 45–50. British Crop Protection Council, Thornton Heath

Asare-Boamah NK, Fletcher RA (1986) Protection of bean seedlings against heat and chilling injury by triadimefon. Physiol Plant 67:353–358

Capell B, Dörffling K (1989) Low temperature induced changes of abscisic acid contents in barley and cucumber leaves in relation to their water status. J Plant Physiol 135:571–575

Flores A, Dörffling K (1990) A comparative study of the effects of abscisic acid and new terpenoid abscisic acid analogues on plant physiological processes. J Plant Growth Regul 9:133–139

Flores A, Grau A, Laurich F, Dörffling K (1988) Effect of new terpenoid analogues of abscisic acid on chilling and freezing resistance. J Plant Physiol 132:362–369

Flores-Nimedez A, Vergara BS, Dörffling K (1990a) Effect of synthetic phytohormone analog on leaf water potential during chilling. IRRN 15:2, 18

Flores-Nimedez A, Dörffling K, Vergara BS (1990b) A new phytohormone analog for preventing rice membrane injury after chilling. IRRN 15:2, 19

Greaves JA, Wilson JM (1986) Assessment of the non-freezing cold sensitivity of wild and cultivated potato genotypes by chlorophyll fluorescence analysis. Potato Res 29:509–520

Grossman K, Jung J (1984) The influence of new terpenoid analogues of abscisic acid on stomatal movement and leaf senescence. Z Acker Pflanzenbau (J Agron Crop Sci) 153: 14–22

Hodgson RA, Raison JK (1984) Chloroplast superoxide (O^{2-}) radical production during low temperature stress in the light. Plant Physiol 75(Suppl): 980

Moll BA, Steinback KE (1986) Chilling sensitivity on *Oryza sativa:* the role of protein phosphorylation in protection against photoinhibition. Plant Physiol 80:420–423

Peeler TC, Naylor AW (1988) A comparison of the effects of chilling on leaf gas exchange in pea (*Pisum sativum* L.) and cucumber (*Cucumis sativus* L.). Plant Physiol 86:143–146

Quarrie SA (1987) Use of genotypes differing in endogenous abscisic acid levels in studies of physiology and development. In: Hoad GV, Lenton JR, Jackson MB, Atkin RK (eds) Hormone action in plant development—a critical appraisal. Butterworths, London, pp 89–105

Rademacher W, Fritsch H, Graebe JE, Sauter H, Jung J (1987) Tetcyclacis and triazole-type plant growth retardants: their influence on the biosynthesis of gibberellins and other metabolic processes. Pestic Sci 21:241–252

Rikin A, Atsmon D, Gitler C (1983) Quantitation of chill-induced release of a tubelin-like factor and its prevention by abscisic acid in *Gossypium hirsutum* L. Plant Physiol 71: 747–748

Rikin A, Atsmon D, Gitler C (1979) Chilling injury in cotton (*Gossypium hirsutum* L.): prevention by abscisic acid. Plant Cell Physiol 20:1537–1546

Rikin A, Blumenfeld A, Richmond AE (1976) Chilling resistance as affected by stressing environments and abscisic acid. Bot Gaz 137:307–312

Schubert J, Roeser K, Grossman K, Sauter H, Jung J (1991) Transpiration-inhibiting abscisic acid analogs. J Plant Growth Regul 10:27–32

Smillie RM, Hetherington SE (1983) Stress tolerance and stress-induced injury in crop plants measured by chlorophyll fluorescence in vivo. Chilling, freezing, ice cover, heat and high light. Plant Physiol 72:1043–1100

Zhang CL, Li PH, Brenner ML (1986) Relationship between mefluidide treatment and abscisic acid metabolism in chilled corn leaves. Plant Physiol 81:699–701