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ORIGINAL ARTICLE

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Interactions between ethylene, gibberellin and abscisic acid regulate emergence and growth rate of adventitious roots in deepwater rice

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Abstract Growth of adventitious roots is induced in deepwater rice (*Oryza sativa* L.) when plants become submerged. Ethylene which accumulates in flooded plant parts is responsible for root growth induction. Gibberellin (GA) is ineffective on its own but acts in a synergistic manner together with ethylene to promote the number of penetrating roots and the growth rate of emerged roots. Studies with the GA biosynthesis inhibitor paclobutrazol revealed that root emergence was dependent on GA activity. Abscisic acid (ABA) acted as a competitive inhibitor of GA activity. Root growth rate on the other hand was dependent on GA concentration and ABA acted as a potent inhibitor possibly of GA but also of ethylene signaling. The results indicated that root emergence and elongation are distinct phases of adventitious root growth that are regulated through different networking between ethylene, GA and ABA signaling pathways. Adventitious root emergence must be coordinated with programmed death of epidermal cells which cover root primordia. Epidermal cell death is also controlled by ethylene, GA and ABA albeit with cell-type specific cross-talk. Different interactions between the same hormones may be a means to ensure proper timing of cell death and root emergence and to adjust the growth rate of emerged adventitious roots.

Keywords Abscisic acid · Adventitious roots · Ethylene · Gibberellic acid · Growth · *Oryza sativa* L.

Abbreviations ABA: Abscisic acid · GA: Gibberellic acid · NBD: Norbornadiene (bicyclo[2.2.1]hepta-2,5-diene) · PAC: Paclobutrazol · PCD: Programmed cell death

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Introduction

Growth of most plants is dependent on a functional root system. The root system consists of embryonic roots and postembryonic roots which include main, lateral and adventitious roots (Fitter 1991). Deepwater rice is a semi-aquatic plant that develops adventitious root primordia at the nodes of the shoot as part of its normal developmental program (Suge 1985; Bleecker et al. 1986). Upon partial submergence of the shoot root primordia emerge. During long-term flooding nodal roots can replace soil-borne roots which become readily anaerobic and are no longer able to supply water and minerals to the shoot. With adventitious roots the transport path between the root tip, which is sensitive to oxygen deprivation, and atmospheric oxygen is shortened and gas exchange is facilitated. Improved gas exchange is further achieved by enhanced formation of aerenchyma in stems and adventitious roots (Justin and Armstrong 1991; Inada et al. 2002).

Zimmerman and Hitchcock (1933) were the first to show that ethylene plays a major role in adventitious root growth. This observation was subsequently confirmed for other plant species including deepwater rice (Suge 1985; Lorbiecke and Sauter 1999), *Rumex palustris* (Peeters et al. 2002), tomato (Phatak et al. 1981) and mung bean (Robbins et al. 1985). Ethylene-insensitive transgenic tobacco plants formed fewer adventitious roots (McDonald and Visser 2003) and ethylene-insensitive transgenic petunia plants displayed reduced adventitious root growth (Clark et al. 1999) supporting the conclusion that ethylene plays a central role in regulating adventitious root growth.

Ethylene accumulates in submerged plants because it is physically trapped in intercellular gas spaces (Musgrave et al. 1972). Some plants such as deepwater rice

promote ethylene biosynthesis during submergence (Raskin and Kende 1984a, 1984b; Kende et al. 1998). These mechanisms put together result in rapid accumulation of physiologically active concentrations of ethylene. Ethylene promoted not only adventitious root growth but also other adaptive responses that are helpful to the plant in coping with flooding-induced oxygen shortage. Submergence-adapted semi-aquatic plants display ethylene-mediated shoot (Kende et al. 1998) or petiole extension (Voesenek et al. 1993; Cox et al. 2004) and formation of aerenchyma (Métraux and Kende 1983; Peeters et al. 2002).

In deepwater rice, emergence and growth of nodal roots was shown to be regulated not only by ethylene but also by gibberellin (GA) (Suge 1985). Involvement of GA in regulating ethylene-mediated growth responses was also shown for shoot growth in *Callitriche* (Musgrave et al. 1972) and rice (Raskin and Kende 1984b), for petiole growth in *Rumex palustris* (Cox et al. 2004), for hyponastic growth in tobacco (Pierik et al. 2004), and for apical hook formation in Arabidopsis (Vriezen et al. 2004). In deepwater rice, ethylene promotes rapid internode elongation by enhancing the amount and the activity of endogenous gibberellic acid (Raskin and Kende 1984b; Hoffmann-Benning and Kende 1992).

Abscisic acid (ABA) is an important signal molecule for abiotic stress adaptation but also acts as a developmental signal. In Arabidopsis, ABA exerts an inhibitory effect on seedling root growth (Beaudoin et al. 2000) and development of lateral roots (De Smet et al. 2003). ABA inhibition of root growth required a functional ethylene signaling cascade and ethylene-insensitive mutants were resistant to ABA inhibition. Ethylene production on the other hand was not the target of ABA action. In contrast, ABA was shown to maintain root growth at low water potential, i.e. drought by inhibiting ethylene synthesis (Sharp 2002). In most plants the action of ethylene is to inhibit growth.

In plants that are not drought-stressed ABA acts as a growth inhibitor in roots and in shoots (Ikeda et al. 2002). The *slender* (*slr*) rice mutant has a constitutive GA-response phenotype with a twofold longer shoot than wild-type plants. The height was similar to wild-type plants that were treated with GA (Ikeda et al. 2002). In the *slr* rice mutant, ABA was shown to suppress shoot elongation indicating that *slr* rice responded normally to ABA. Because ABA can suppress the effect of the *slr1* mutation it was suggested that ABA is likely to be involved in GA signaling.

Ethylene, GA and ABA interact in different ways to achieve different growth responses and to control developmental processes. The upward movement of young petioles of *Rumex palustris* (Cox et al. 2004), the so-called hyponastic response, allows submerged plants to bring the leaf blade above the water surface (Voesenek et al. 2003). Varying interactions between ethylene, GA, ABA and auxin control initiation and speed of petiole and hyponastic growth upon submergence. Genetic studies support physiological data and suggest that overall sensitivity of a plant cell to a hormone is at least partially established by the interplay of several hormones (Trewavas 1992). One interesting and mostly unanswered question remains how various tissues manage to respond differently to the same hormonal cocktail. We can gain a progressively better understanding of how various hormonal responses are coordinated by elucidating interactions between hormones and lag phases for each response.

Our current work established that at least three hormones, namely ethylene, GA and ABA, are involved in regulating adventitious root growth. Our study aimed at elucidating the interplay between these hormones in controlling different growth phases, i.e. root emergence and root elongation.

Materials and methods

Plant material and growth conditions

Deepwater rice plants (*Oryza sativa* L., Pin Gaew 56) were grown as described (Sauter 1997). Seeds of the rice cultivar Pin Gaew 56 were originally obtained from the International Rice Research Institute (Los Banos, Philippines). Stem sections containing the third node were prepared from 12- to 14-week-old plants. To obtain stem sections the shoot was cut 2 cm below the third node and 20 cm above this first cut (Mergemann and Sauter 2000). Stem sections were placed in a 150 ml beaker containing 20 ml of aqueous solution of hormones or growth inhibitors. Plastic cylinders covered the beakers with the stem sections to assure high humidity. All experiments were performed at 27°C under permanent light at 150 μmoles m^{-2} s^{-1} .

Hormone and inhibitor treatment

Aqueous solutions of ethephon (2-chloroethanephosphoric acid), gibberellic acid A_3 (GA_3) and ABA were used at the final concentrations indicated in the figures. Ethephon (Sigma, Steinheim, Germany) was prepared from liquid stock. Previous studies showed that ethephon had an optimal effect on adventitious root penetration at 150 μM (Lorbiecke and Sauter 1999). The penetration rate of stem sections treated with 150 μM ethephon was comparable to the penetration rate observed with partially submerged intact plants. Higher concentrations of ethephon were toxic. To study the synergistic effects of GA and ethylene, suboptimal concentrations of 15 or 50 μM ethephon were chosen.

GA_3 and ABA crystals (Sigma, Steinheim, Germany) were dissolved in water to yield 100-fold aqueous stocks. To inhibit GA biosynthesis (Gianfagna et al. 1998; Rademacher 2000), 10- to 11-week-old plants were pretreated for 8 days in hydroponic fertiliser containing 2 μM paclobutrazol (PAC) (Duchefa, The Netherlands). PAC powder was dissolved directly in hydroponic fer-

tilizer. Treatment with 2 µM PAC was continued during incubation with hormones. In this case, PAC was dissolved in water and added to the hormone solution from a 100-fold aqueous stock solution. 2,5-Norbornadiene (bicyclo[2.2.1]hepta-2,5-diene; NBD) was used to inhibit ethylene activity. It was applied to stem sections at 1 or 5 µl/l in the gas phase.

Measurement of adventitious root penetration and root length

Stem sections were treated for 48 h with various hormone and inhibitor combinations at the concentrations indicated. Subsequently the percentage of roots which were penetrated through the epidermis was determined. The total number of adventitious root initials was taken as 100%. The lengths of the penetrated roots were measured with a ruler. The average length of all roots was calculated whereby non-penetrated roots were counted as having a length of 0 mm. Statistical analysis were done by using the Mann – Whitney test (*U*-test). Significantly different values at $P \leq 0.05$ were marked with different letters.

Results

Gibberellin promotes ethylene-induced adventitious root emergence and growth rate

Suge (1985) showed that nodal root growth in deepwater rice is under the control of ethylene and GA. We studied the effect of both hormones in more detail, focussing on two aspects of adventitious root growth namely on root penetration and on root elongation rate. Rice stem sections were treated for 48 h with ethephon at different concentrations in the presence or absence of GA_3 (Figs. 1a, 2a). Increasing concentrations of ethephon resulted in significantly higher percentages of penetrated roots with the highest concentration of 150 µM ethephon applied leading to 76.2% penetrated roots (Fig. 1a). In the presence of 3 µM GA_3 the dose response curve of ethylene was shifted to lower concentrations. A rate of 50% root penetration was achieved with 30 µM ethephon or with 4.5 µM ethephon combined with 3 µM GA_3 (Fig. 1a).

Suge (1985) and Lorbiecke and Sauter (1999) previously showed that GA_3 when applied to rice stems by itself had little or no effect on adventitious root growth. Our current work expanded these studies to show the effect of increasing concentrations of GA_3 in combination with a suboptimal ethephon concentration of 5 µM on root penetration (Fig. 1b). Incubation with GA_3 by itself confirmed earlier results that GA even at high concentrations resulted in only limited root penetration. A maximal value of 18.6% was achieved at 100 µM GA_3 as compared to 76% root penetration achieved with 150 µM ethephon (Fig. 1a). When GA_3 was applied together with 5 µM ethephon a synergistic effect on root penetration rate was observed over a wide range of GA_3 concentrations (Fig. 1b).

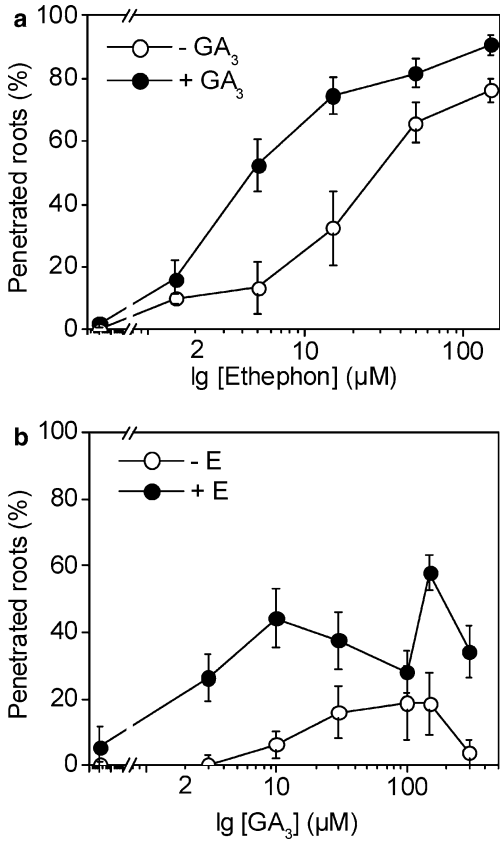

Fig. 1 Ethephon-induced emergence of adventitious roots is promoted by GA in a synergistic manner while GA by itself has little effect. Percentages of penetrated adventitious roots were calculated after 48 h of treatment with **a** 0–150 µM ethephon with or without 3 µM GA_3 and **b** 0–300 µM GA_3 in the presence or absence of 5 µM ethephon (E). Averages ± SE were calculated from two to three independent experiments

Root growth was stimulated increasingly by increasing ethephon concentrations (Fig. 2a). Combining ethephon with 3 µM GA_3 resulted in a synergistic growth promoting effect at mostly all ethephon concentrations applied. As with root penetration, application of 3 µM GA_3 in addition to ethephon shifted the dose response curve to lower ethephon concentrations (Fig. 2a). After 48 h an average root length of 2 mm was achieved either with approximately 40 µM ethephon or with 6 µM ethephon combined with 3 µM GA_3. Root growth rate was hardly induced by treatment with physiologically relevant concentrations of GA_3. Roots that did penetrate with GA_3 remained very short (Fig. 2b). Incubation with 5 µM ethephon had little effect on root growth whereas combining 5 µM ethephon with GA_3 at various concentrations resulted in an upto 11-fold increase in root length (Fig. 2b). In summary, penetration and growth of adventitious roots were induced by ethylene and these responses were strongly promoted by GA resulting in a synergistic effect of both hormones.

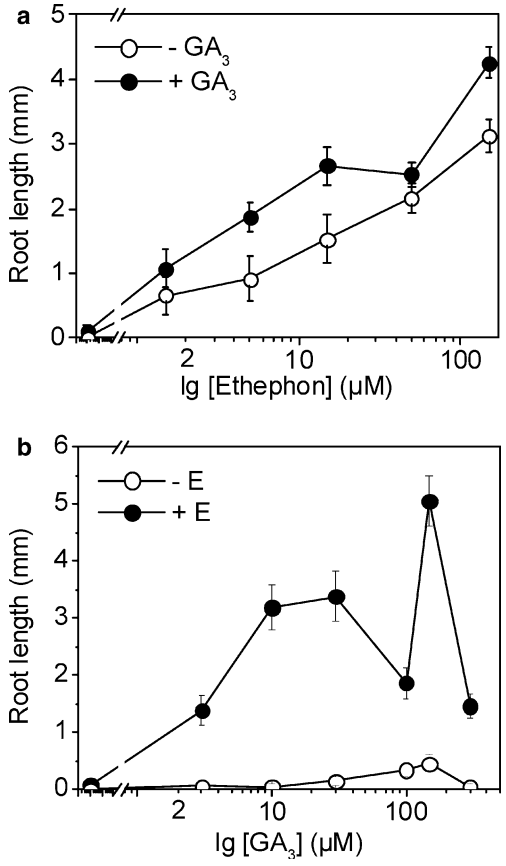

Fig. 2 Gibberellic acid promotes ethephon-induced root growth, but has little effect on root growth rate in the absence of ethylene. Lengths of adventitious roots were determined from the same stem sections analyzed in Fig. 1. Lengths of roots were measured after 48 h of treatement with **a** 0–150 μM ethephon with or without 3 μM GA_3 and **b** 0–300 μM GA_3 in the presence or absence of 5 μM ethephon (E). Results are averages ± SE from two to three independent experiments

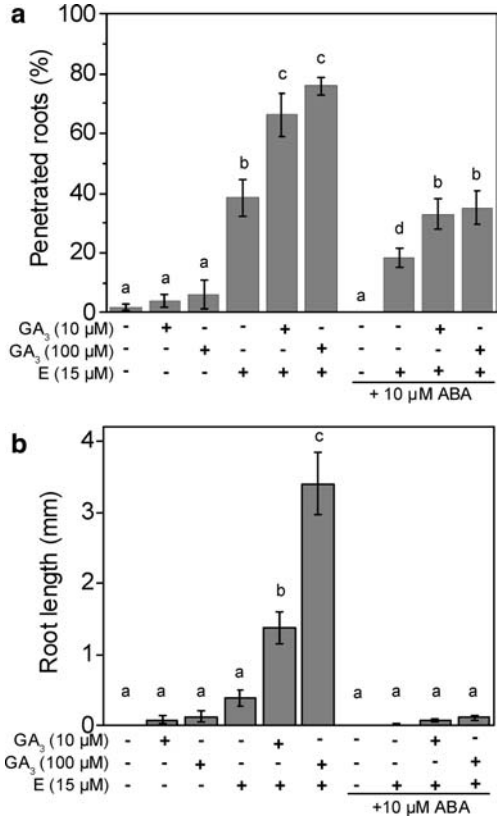

Fig. 3 Abscisic acid inhibits ethylene-induced and GA-promoted emergence and growth of adventitious roots to different degrees. **a** Percentages of penetrated roots after treatment with different combinations of ethephon (E), GA_3 and ABA for 48 h at the concentrations indicated. **b** Lengths of roots were determined after treatment for 48 h as in (**a**). Results are averages ± SE from two independent experiments. Values with different letters are significantly different from each other at $P \leq 0.05$ (Mann – Whitney test)

ABA inhibits ethylene-induced and GA-promoted penetration and growth of adventitious roots to different degrees

In flooding-tolerant semi-aquatic plants, such as deepwater rice, the balance of ethylene, GA and ABA is altered upon submergence and this altered balance was shown to control various adaptive responses. We tested the possibility that root penetration and adventitious root growth may be controlled by ABA as well in addition to being regulated by ethylene and GA. We treated rice stem sections for 48 h with a combination of ethephon, GA_3 and ABA (Fig. 3). GA_3 was used at concentrations of 10 or 100 μM and caused 6.1% or less roots to penetrate (Fig. 3a). Ethephon at a concentration of 15 μM led to a penetration rate of 38.5% after 48 h. Application of 15 μM ethephon and 10 or 100 μM GA_3 resulted in the already described synergistic increase in penetration rate of 66.4 or 75.9% penetrated roots, respectively. In the presence of 10 μM ABA root penetration induced by ethephon or by ethephon in combination with GA_3 was reduced to about half (Fig. 3a). Inhibition of root emergence by ABA was partially overcome by addition of 10 μM GA_3. A tenfold higher GA_3 concentration did not further alleviate ABA inhibition indicating that ABA did not compete exclusively with GA activity.

The average adventitious root length was 0.4 mm after treatment with 15 μM ethephon for 48 h, 1.4 mm with 15 μM ethephon and 10 μM GA_3 and 3.4 mm with 15 μM ethephon and 100 μM GA_3 (Fig. 3b). When ABA was applied in addition to ethephon the growth rate was reduced to near zero (Fig. 3b). Growth inhibition by ABA was not reversed even by high levels of GA_3, indicating that ABA acted as a strong inhibitor of ethylene-induced and GA-promoted root growth. The effect of ABA on ethylene-induced root penetration as well as root growth rate was dose-dependent between 1 and 100 μM ABA (data not shown).

Paclobutrazol inhibits growth but not penetration of adventitious roots

Paclobutrazol was used to inhibit GA biosynthesis and thus reduce endogenous GA levels. Plants were treated for 8 days with 2 μM PAC. This pretreatment inhibited

growth of the youngest internode which is known to depend on GA (Raskin and Kende 1984b; Hoffmann-Benning and Kende 1992) thus indicating that endogenous GA levels were reduced (data not shown).

Incubation of control stem sections with 150 µM ethephon caused 80% of root initials to penetrate (Fig. 4a) confirming earlier results (Fig. 1). In stems that were obtained from PAC-treated plants a nearly identical root penetration rate of about 80% was observed with 150 µM ethephon. Addition of 30 µM GA_3 in combination with 150 µM ethephon increased root penetration rate to about 90% in the absence as well as in the presence of PAC (Fig. 4a). Thus long-term PAC treatment did not reduce the ability of root initials to penetrate in response to ethylene. A small but reproducible effect of PAC on root penetration rate was observed in response to GA. In non-PAC treated stems GA_3 did not induce root penetration over control levels whereas in stems that were obtained from PAC-treated plants more roots penetrated in the presence of GA_3 compared to controls without PAC or controls without GA_3 (Fig. 4). In conclusion, long-term PAC treatment increased the penetration rate of root primordia in response to exogenously applied GA.

At a suboptimal concentration of 15 µM ethephon in the presence of PAC root penetration rates were saturated with 30 µM GA_3. In the absence of PAC penetration rates slightly increased from 76% with 30 µM GA_3 to around 88% with 100 or 300 µM GA_3 (Fig. 4b). Higher penetration rates at lower GA concentrations may be a result of increased responsiveness of adventitious roots towards GA after PAC treatment. A similar increased responsiveness to GA was observed for epidermal cells which cover adventitious roots in deepwater rice and which undergo programmed cell death (PCD; Steffens and Sauter, 2005).

Once roots emerged from the node, root growth rate was dependent on GA levels. The average root length was 4.4 mm after treatment with 150 µM ethephon for

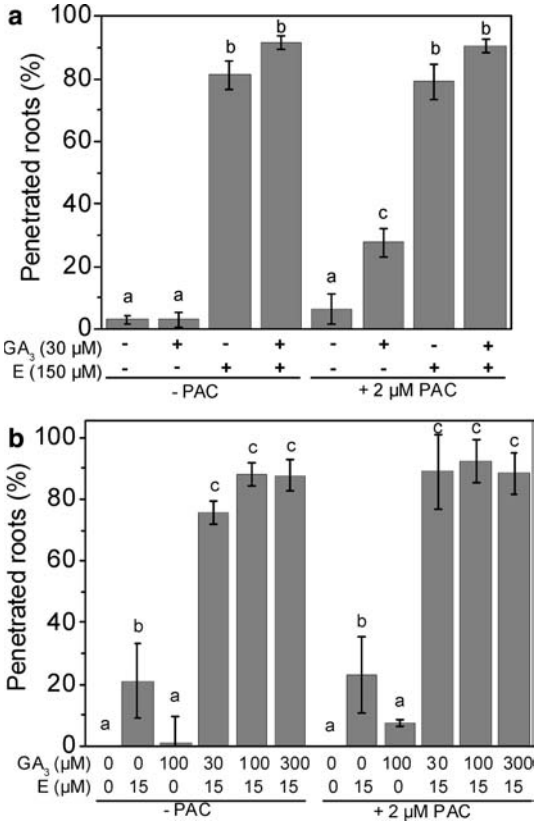

Fig. 4 Paclobutrazol does not inhibit penetration of adventitious roots. Plants were pretreated with 2 µM PAC for 8 days and PAC treatment was continued during hormone treatment. As a control, plants were grown without PAC. Percentages of penetrated roots were determined after treatment for 48 h **a** with or without 150 µM ethephon (E) and with or without 30 µM GA_3 and **b** with or without 15 µM ethephon (E) at 0 µM, 30 µM, 100 µM or 300 µM GA_3. Results are averages ± SE from three independent (**a**) or one (**b**) experiment(s). Values with different letters are significantly different from each other at $P \leq 0.05$ (Mann – Whitney test)

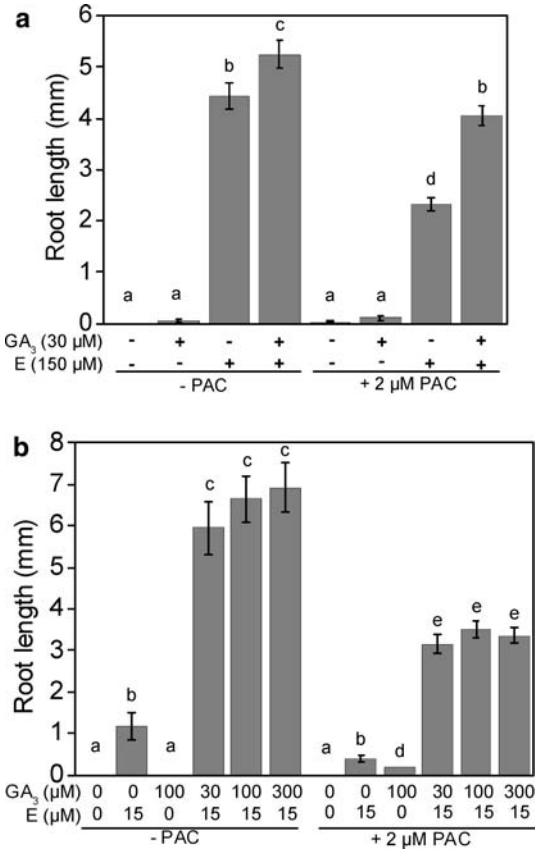

Fig. 5 Inhibition of root growth by PAC is partially reversed by exogenous GA. Plants were pretreated with 2 µM PAC for 8 days and PAC treatment was continued during hormone treatment. As a control, plants were grown without PAC. Lengths of roots were determined after 48 h from the same stem sections analyzed in Fig. 4a and b, respectively. Stem sections were treated **a** with or without 150 µM ethephon (E) and with or without 30 µM GA_3 and **b** with or without 15 µM ethephon (E) at 0 µM, 30 µM, 100 µM or 300 µM GA_3. Results are averages ± SE from three independent (**a**) or one (**b**) experiment(s). Values with different letters are significantly different from each other at $P \leq 0.05$ (Mann – Whitney test)

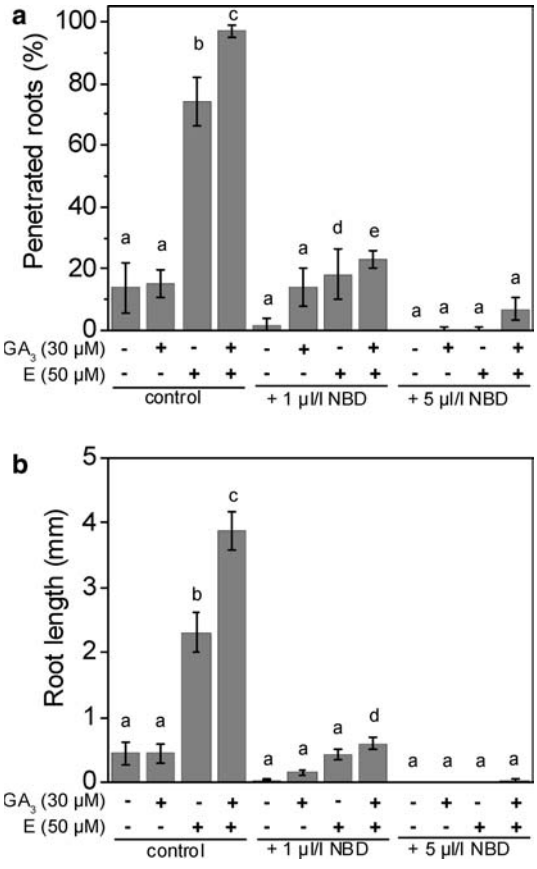

Fig. 6 Norbornadiene (bicyclo[2.2.1]hepta-2,5-diene) inhibits penetration and growth of adventitious roots. Stem sections were treated without (control) or with 1 µl/l or 5 µl/l NBD. **a** Percentages of penetrated roots were determined after 48 h of treatment with or without 50 µM ethephon (E) in the presence or absence of 30 µM GA_3. **b** Lengths of roots were determined from the same stem sections as in (**a**). Results are averages ± SE from four independent experiments. Values with different letters are significantly different from each other at $P \leq 0.05$ (Mann – Whitney test)

48 h in the absence of PAC and 2.3 mm after treatment with 150 µM ethephon for 48 h in the presence of PAC. Thus PAC inhibited ethephon-induced root growth by about 50% and this root growth was largely restored by application of GA_3 at 30 µM (Fig. 5a). How about reversibility of PAC inhibition of GA-promoted ethephon-induced root growth by supplementation with GA_3? To answer this question we applied a low level of 15 µM ethephon in combination with 30, 100 or 300 µM GA_3 in the presence or absence of PAC (Fig. 5b). Ethephon at 15 µM resulted in an average root length of 1.2 mm which was reduced to 0.4 mm in the presence of PAC. When PAC-treated stems were supplemented with GA_3 in addition to 15 µM ethephon root growth was increased to approximately 3 mm which was more than that with ethephon alone but less than the 6 – 7 mm achieved with 15 µM ethephon and GA_3 in the absence of PAC (Fig. 5b). Growth of PAC-treated stems did not exceed 3.4 mm even at high GA_3 concentrations, indicating that the effect of PAC was not fully reversible by application of GA_3. Obviously PAC affected not only GA biosynthesis. Rather it appeared that the responsiveness to exogenously supplied GA_3 was altered as well possibly as a consequence of lowered GA levels. Roots from PAC-treated stems showed reduced sensitivity towards exogenously supplied GA_3 and a lower maximal growth rate as compared to control roots.

Experiments with PAC as inhibitor of GA biosynthesis revealed that adventitious root growth proceeds in two stages which are regulated differently by GA. Initiation of root growth was not limited by endogenous GA whereas the growth rate of emerged roots was dependent on endogenous GA levels and on the responsiveness of roots towards exogenously supplied GA. At low internal GA levels as found in non-submerged plants adventitious root growth would not be favored.

GA-promoted root emergence and root extension rate are both dependent on ethylene signaling

In order to test whether GA promotion of root growth could bypass ethylene signaling we employed NBD as a competitive inhibitor of ethylene action (Bleecker et al. 1987). We showed previously that ethephon-induced adventitious root growth was fully inhibited by NBD (Lorbiecke and Sauter 1999). Root growth was analyzed after treatment with 50 µM ethephon and 30 µM GA_3 in the presence or absence of NBD (Fig. 6). Root emergence and root growth rate were induced by ethylene and further promoted by GA_3 as shown above. Treatment with 1 µl/l NBD resulted in strong inhibition and treatment with 5 µl/l NBD (Fig. 6) and 50 µl/l NBD (data not shown) in full inhibition of root penetration and growth. Thus promotion of root emergence and growth by GA were dependent on ethylene perception.

Discussion

Adventitious roots are induced to grow at nodes when deepwater rice plants are partially submerged. These secondary roots replace or support the main root system that can rapidly become disfunctional due to limited oxygen supply in flooded soil. Adventitious root growth is induced by ethylene as are many other responses to hypoxia. Suge (1985) showed that GA also plays a role in regulating adventitious root growth in rice. GA applied by itself, slightly increased the number of roots that emerged per node and the length of the longest root. When GA was applied together with ethylene a strong synergistic effect on root number and root length was observed. Our experiments essentially confirmed these results whereby both emergence of adventitious roots and growth rate were induced by ethylene and enhanced by GA in a synergistic manner.

When endogenous GA levels were lowered with the GA biosynthesis inhibitor PAC penetration of roots was

not impaired. Rather PAC-treated root primordia showed the same or slightly increased penetration rates in response to ethylene and GA or in response to GA applied in the absence of ethylene. The results indicated that the responsiveness of adventitious root primordia toward exogenous GA was increased after long-term PAC treatment. We therefore concluded that root emergence was determined by GA activity rather than by GA concentration.

We identified ABA as a hormone which negatively controls root emergence. Penetration of adventitious roots was inhibited by about 50% with 10 μM ABA. ABA reduced both ethylene-induced as well as GA-promoted root penetration. The idea that GA and ABA might act on roots in a competitive manner was tested by applying increasing concentrations of GA to ethylene and ABA-treated roots. Penetration rates increased significantly when 10 μM GA_3 were added to stems that were incubated with ethylene and ABA. They were restored with 10 μM GA_3 to the penetration rate obtained with 15 μM ethephon in the absence of ABA. When the concentration of GA_3 was raised to 100 μM the penetration rate did not increase further. Thus ABA activity on root penetration appeared to be at least in part in competition with GA activity.

Treatment of roots with a combination of ethylene and GA resulted in higher growth rates than those observed with ethylene alone revealing a synergistic effect of both hormones as was observed for PCD (Steffens and Sauter, 2005) and adventitious root penetration. In contrast to root emergence which was dependent on GA activity, growth of emerged roots was dependent on GA concentration. Application of GA by itself had little effect on root growth. However, when endogenous GA levels were lowered with the GA biosynthesis inhibitor PAC, ethylene-dependent root growth was reduced. PAC also inhibited internodal growth to the same degree as root growth indicating that it effectively reduced endogenous GA levels (data not shown). PAC-inhibited root growth was partly compensated by application of exogenous GA. These results indicated that endogenous GA acted to promote growth induced by ethylene. A similar observation was made in tomato where exogenously applied GA_3 had no effect on root elongation, but treatment with the GA biosynthesis inhibitor tetcyclasis resulted in shorter roots. This inhibition was partially overcome by exogenously applied GA_3. The authors concluded that root growth may be controlled by GA and that endogenous GA levels in roots were at or near optimal levels (Fellner et al. 2001; Tanimoto 1991).

Growth of penetrated roots was induced by ethylene and was further promoted by GA. Treatment with NBD abolished root growth indicating that ethylene perception was required for roots to elongate. ABA acted as potent inhibitor of ethylene-induced root growth and growth inhibition was not reversed by GA when added in ten-fold excess. Thus in contrast to what we observed with root emergence, ABA appeared to exert a dominant effect on ethylene signaling. Similarly, in Arabidopsis, root growth was inhibited by 10 μM ABA. Using ethylene signaling mutants it was shown for Arabidopsis that a functional ethylene signaling pathway was required for ABA signaling (Beaudoin et al. 2000; Ghassemian et al. 2000). In deepwater rice, ABA inhibited ethylene activity. Additional interaction of ABA with the GA-specific signaling pathway can, however, not be excluded.

We found evidence that root penetration was dependent on GA activity whereas elongation growth of emerged roots was dependent on GA concentration. This observation led us to conclude that root cells alter their responsiveness towards GA after roots have penetrated through the epidermis. Hyponastic growth of *Rumex palustris* petioles was shown to proceed in defined stages which are controlled by different hormones (Cox et al. 2004). Initiation of petiole growth was promoted by ethylene and auxin whereas growth rate was positively influenced by ethylene and GA. At both stages ABA acted as inhibitory signal.

Ethylene and GA were identified as root penetration and root growth promoting hormones. Since the effects of GA and ethylene were not additive but synergistic, shared signaling components must be predicted. One question that we attempted to answer was which signaling pathway was mandatory for root growth to occur? GA by itself was nearly ineffective in promoting root penetration or growth. When roots were treated with NBD, an inhibitor of ethylene perception neither ethylene (Lorbiecke and Sauter 1999) nor GA were able to promote root penetration or growth. Therefore it appeared that GA activity on adventitious roots required ethylene signaling.

This result would be consistent with the view that GA acts upstream of ethylene perception possibly on ethylene synthesis. However, GA by itself cannot induce roots to grow whereas ethylene can do so. Therefore, it seems unlikely that GA acts by promoting ethylene synthesis because we then would expect to see root growth with GA. Alternatively it is conceivable that GA acts downstream of the ethylene receptor and GA activity requires activated ethylene signaling achieved through ethylene binding to its receptor.

At which point the GA and ethylene signaling pathways merge in the control of adventitious root growth was not addressed. However DELLA proteins were identified as key components of GA signaling (Fu et al. 2002; Fu and Harberd 2003; Fleet and Sun 2005). DELLA proteins are transcriptional regulators with repressing activity (Fu et al. 2002; Fu and Harberd 2003). In Arabidopsis it was shown that promotion of root growth by GA was achieved through degradation of DELLA proteins which otherwise act to suppress growth (Fu et al. 2002; Fu and Harberd 2003). Furthermore, asymmetric growth of the hypocotyl which is responsible for apical hook formation in Arabidopsis seedlings depends on ethylene and was also recently shown to require GA. Elevated ethylene levels resulted

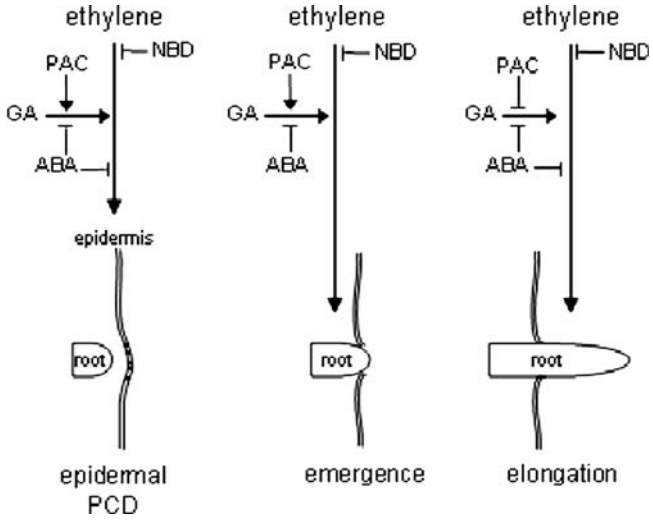

Fig. 7 Model summarizing our current view on hormonal regulation of epidermal PCD (see Steffens and Sauter, 2005), root penetration and root elongation growth in deepwater rice. PCD is dependent on ethylene signaling, is promoted by GA activity and is fully inhibited by ABA likely through interaction with ethylene and possibly through interaction with GA signaling. Root emergence is dependent on ethylene signaling, is promoted by GA activity and is partially inhibited by ABA possibly through interference with GA activity. Root growth is dependent on ethylene signaling, promoted by GA in a concentration-dependent manner and is fully inhibited by ABA likely through interaction with ethylene and possibly GA signaling similar to PCD

in accumulation of a DELLA protein in the nucleus (Vriezen et al. 2004) indicating that DELLA proteins can be a common target of ethylene and GA signaling possibly through targeted proteolysis or altered subcellular localization.

We recently described regulation of cell death that affects cells of the epidermis which cover nodal root primordia (Steffens and Sauter 2005). PCD is induced by ethylene, promoted by GA and is effectively blocked by ABA, a regulation pattern that resembles the pattern observed with root elongation growth. For PCD and root emergence, responsiveness towards GA was altered as a consequence of an altered GA concentration. This was not observed for root elongation growth. Our studies thus revealed that root penetration and elongation growth can be viewed as distinct stages that are regulated differentially by ethylene, GA and ABA (Fig. 7).

Expansins are known to mediate cell wall loosening and are believed to be major determinants of cell expansion (Cosgrove 2000). Expansins are encoded by multi-gene families. In *Rumex*, it was shown recently that expansin genes are expressed differentially not only in different root types but also along the lengths of adventitious roots (Colmer et al. 2004). It is conceivable that individual expansin genes are regulated differently by ethylene, GA and ABA and that this differential activation preferentially affects either root emergence or adventitious root growth rate. In support of this hypothesis Colmer et al. (2004) found in *Rumex* that hypoxia which alters the hormonal balance and growth also resulted in altered expansin gene expression.

In summary, emergence and growth of adventitious roots are both controlled by ethylene signaling which is enhanced by GA and inhibited by ABA. Regulation of root emergence by GA is dependent on concentration and activity of GA whereas root elongation is largely dependent on GA concentration. ABA appears to act mainly on GA signaling in inhibiting root emergence while it appears to interfere with the ethylene signaling pathway in inhibiting root elongation growth possibly in addition to inhibition of GA signaling. Since ABA is a powerful inhibitor of root elongation up-regulation of ABA levels in the plant may be a means to rapidly stop root elongation.

Thus three events can be distinguished at the node, i.e. epidermal PCD, root emergence and root elongation (Fig. 7). All three events are dependent on ethylene signaling but the ethylene responses are subject to adjustments by GA and ABA in different ways. What is the advantage of these distinct regulatory pathways to the plant? One aspect may be coordination between epidermal cell death and root emergence. Death of epidermal cells is thought to allow penetration of roots without damaging the root tip. What would happen if epidermal cells underwent PCD but adventitious roots did not penetrate? If root primordia did not grow through the epidermal layer the plant surface would be unprotected at the sites of cell death similar to wounding. Entry or loss of salts or water and invasion by pathogens would be facilitated. Thus once epidermal cells are dead root primordia may be required to fill the gap left by the dead cells. Initiation of cell death should therefore, be coupled to initiation of root emergence. This must happen in a timely fashion because root primordia should not grow before epidermal cells have given way. We know that both events, PCD and root emergence, depend on ethylene signaling which is modulated by GA activity in a similar manner. The two processes are thus positively regulated by the same interactions between ethylene and GA. ABA on the other hand acts differentially on PCD and root emergence. It inhibits PCD more efficiently than root emergence. It was described that ABA levels decrease severalfold in semi-aquatic plants within the first few hours of submergence (Hoffmann-Benning and Kende 1992; Azuma et al. 1995; Cox et al. 2004). In the growth-responsive region of deepwater rice stems a ten-fold decline in ABA concentration was observed after one day of submergence (Hoffmann-Benning and Kende 1992; Azuma et al. 1995). PCD is stringently controlled by ABA (Steffens and Sauter, 2005), more so than is root emergence (this study). Reducing ABA concentration may in turn relieve inhibition of PCD more effectively and more rapidly than inhibition of root emergence. The difference in responsiveness to ABA may thus be one mode through which the plant assures that PCD occurs prior to root emergence.

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