Article Addendum

Examination of two lowland rice cultivars reveals that gibberellin-dependent early response to submergence is not necessarily mediated by ethylene

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Summary

Using two lowland rice (*Oryza sativa* L.) cultivars we found that in both cases submerged-induced elongation early after germination depends on gibberellins (GAs). Submergence increases the content of the active GA₁ by enhancing the expression of GA biosynthesis genes, thus facilitating the seedlings to escape from the water and preventing asphyxiation. However, the two cultivars differ in their response to ethylene. The cultivar Senia (short), by contrast to cultivar Bomba (tall), does not elongate after ethylene application, and submerged-induced elongation is not negated by an inhibitor of ethylene perception. Also, while ethylene emanation in Senia is not altered by submergence, Bomba seedlings emanate more ethylene upon de-submergence, associated with enhanced expression of the ethylene biosynthesis gene *OsACS5*. The cultivar Senia thus allows the possibility of clarifying the role of ethylene and other factors as triggers of GA biosynthesis enhancement in rice seedlings under submergence.

To Grow or not to Grow, that is the Question

Many plants (e.g. from *Rumex* and *Echinochloa* genera) are able to survive in flooded soils as a result of faster growth induced by gibberellins (GA), thus keeping their leaves in the air and preventing asphyxiation. This is also the strategy used by deepwater rice, grown in heavy rain areas from southeastern Asia. By contrast, lowland rice cultured in regions subjected to occasional and temporal flooding, that do not grow fast enough to escape from flooding, may benefit from shoot elongation restriction under these conditions because this allows saving energy reserves until resuming development following eventual de-submergence. This kind of rice tolerance to submergence is conferred by an ethylene responsive factor (encoded by the gene *Sub1A*), that reduces...
GA-inducible expression under submergence by increasing the levels of the GA signalling repressors SLR1 and SLRL1\(^8,9\).

Lowland rice is germinated in the field under a layer of water in many countries (e.g. in most Mediterranean regions), but at later developmental stages only the basal part of the plant is maintained under water. In this case, increased elongation capacity of the seedlings is a clear advantage to help reaching contact with air and start active photosynthesis as soon as possible. This is opposite to the situation described above, where reduced growth of adult plants under water facilitates eventual growth resumption after temporary flooding receding.

Submergence Induces Elongation of Lowland Rice Seedlings by Increasing Their Content in Active GA\(_1\) through Alteration of GA Metabolism

A comparison study of two lowland rice cultivars grown in the Comunidad Valenciana, Spain (Senia, low, and Bomba, tall), showed that early growth after germination, and also that the enhanced-induced elongation under water depend on GA, in agreement with results obtained using other cultivars\(^4\). This conclusion was based on the observation that rice elongation was reduced by paclobutrazol (an inhibitor of GA biosynthesis) and that this effect was counteracted by application of GA\(_3\). More important, enhanced elongation was associated with an increase on GA\(_1\) content, the purported active GA in rice\(^10\). The contents of GA\(_{53}\) and GA\(_{19}\), but not of GA\(_{20}\), immediate GA\(_1\) precursors\(^{10,11}\), also increased. The submergence increases GA\(_1\) content in the seedlings as a result of its effect on GA metabolism, mainly by enhancing the expression of OsGA20ox1 and -2, and of OsGA3ox2 (GA biosynthesis genes). It is no clear whether the decrease of GA inactivating genes (OsGA2ox) has also a role in the process.
Submergence-Induced Elongation is not Mediated by Ethylene in the Lowland Rice Cultivar Senia

It is known that in the case of deepwater rice stem elongation under submergence is due to the increase of ethylene content in the tissues (due to less diffusion under water but also to enhanced new synthesis)^2,3,4,12^, that stimulates GA biosynthesis. A similar conclusion was obtained in the case of the tall lowland rice cultivar IR36^4^. We therefore investigated whether ethylene was also active in enhancing lowland rice seedling elongation of the Bomba and Senia cultivars. Interestingly, while Bomba seedling elongated in response to ethylene or 1-aminocyclopropane-1-carboxylic-acid (ACC; an ethylene precursor) application, as expected, Senia seedlings did not. Moreover, submerged-induced elongation in Bomba was negated in the presence of 1-methylcyclopropene (1-MCP; an ethylene perception inhibitor), but not in Senia. These results suggested that ethylene is not a factor triggering GA-induced elongation upon submergence in Senia. This hypothesis was also supported by the observation that while ethylene emanation increased about twice in Bomba seedlings following de-submergence it was not affected in Senia (where the rate of ethylene emanation was similar to that found in Bomba submerged seedlings). The increase of ethylene emanation in Bomba was associated with rapid enhanced expression of the ethylene biosynthetic gene OsACS5 upon submergence, an effect not found in cultivar Senia. The expression of other genes involved in ethylene biosynthesis (OsACSI, -2 and -3 and OsACO1) did not change. In agreement with previous observations in deep-water rice^13,14^, our results suggest that in Bomba OsACC5 plays an important role in early submergence-induced ethylene biosynthesis.
Conclusions

As summarized in Fig. 1, submerged-induced seedling elongation of lowland rice is a consequence of increased GA\(_1\) content due to enhanced expression of several GA biosynthesis genes (\textit{OsGA20ox1}, \textit{OsGA20ox2} and \textit{OsGA3ox2}). This effect, as expected, seems to be mediated by ethylene in the tall cultivar Bomba, where submergence also enhances expression of the ethylene biosynthesis gene \textit{OsACS5} and higher ethylene emanation. By contrast, in the short cultivar Senia submergence-induced elongation does not seem to be mediated by ethylene. In this case, the signal triggering GA biosynthesis may be the acidity produced by CO\(_2\) accumulation, as occurs in \textit{Potamogeton pectinatus}\(^{15,16}\). Another factor that might also act as a triggering signal for submergence-induced elongation is hypoxia, because sheath elongation of both Senia and Bomba seedlings was enhanced when grown in a 3% O\(_2\) atmosphere.

Transgenic rice plants expressing antisense \textit{OsEin2}, a positive regulator of the signal ethylene pathway, have reduced stature and display higher rate of ethylene biosynthesis\(^{17}\). In \textit{Arabidopsis thaliana}, blocking the ethylene-signalling pathway also enhances ethylene biosynthesis rate\(^{18}\). Therefore, the insensitivity of Senia to ethylene and 1-MCP, and its higher ethylene biosynthesis compared to Bomba, suggests that ethylene reception and/or signal transduction pathway may be impaired in that cultivar, thus affecting ethylene metabolism.
References


Figure legends

Figure 1. Scheme summarizing the different mechanisms explaining the submergence-induced elongation in two lowland rice cultivars, Bomba (tall) and Senia (short). In both cases, elongation is the result of an increase of active GA (GA$_1$) biosynthesis due to enhanced expression of GA biosynthesis genes (OsGA20ox1, OsGA20ox2 and OsGA3ox2). In the case of Bomba this is induced, at least partially, by up-regulation of OsACS5 expression leading to increase of ethylene biosynthesis. Increase of ethylene biosynthesis is due to enhanced OsACS5 expression. In the case of Senia, submergence-induced elongation does not depend on ethylene, and the GA-mediated response is triggered by an still unknown mechanism, probably involving increase of acidity and/or hypoxia.
Figure 1

Submergence (acidity, hypoxia)

cv. Bomba

↓

Increase of ethylene biosynthesis (OsACS5)

cv. Bomba

↓

Increase of GA$_1$ biosynthesis (OsGA20ox1, OsGA20ox2 and GA3ox2) and response

↓

Elongation