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EFFECT OF ANAEROBIC ENVIRONMENT ON GERMINATION AND GROWTH OF RICE AND WHEAT: ENDOGENOUS LEVELS OF ABA AND IAA

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Summary. Indoleacetic acid and abscisic acid are present in cereal seeds. It is believed that they play roles in seed maturation and germination. Modification of environmental conditions can inhibit or modify germination and seedling elongation. Our aim is to analyse the role played by IAA and ABA during the anoxia seedlings growth in lack of oxygen comparing rice, *Oryza sativa*, the only crop plant able to germinate in absence of oxygen, to wheat, *Triticum aestivum*. Seeds of both, rice and wheat, showed to contain either IAA and ABA stored in free and conjugated forms, the later was predominantly. The examine of the metabolism of the stored IAA and ABA during the first week of rice aerobic germination showed that IAA- and ABA-conjugates were hydrolysed and transported to coleoptiles and roots. During anoxic germination of rice the hydrolysis of IAA conjugates occurred to a reduced low quantity, while ABA stored forms were hydrolysed and rapidly released in the growth medium. Wheat did not germinate in anaerobic conditions, so wheat seedling germinated in air were then transferred to anoxic environment. In this condition the seedling growth was blocked. IAA and ABA, in free form, were present either in coleoptile and root in relevant amount, compared to rice tissues, after 2 days of air germination. Both substances stopped to increase when wheat seedling were transferred to anoxia. No release of ABA in the medium was detected.

The results from the comparison between anoxia tolerant and intolerant cereal species give indication that the endogenous level of free IAA is not strictly correlated with anaerobic coleoptile elongation capacity, while the ability to decrease the endogenous level of ABA coupled with the ability to excrete ABA in the growth medium could be seen as an adaptive mechanism to allow elongation of anaerobic rice coleoptile.

Key words**:** abscisic acid, anaerobic stress, germination, indoleacetic acid, rice, wheat.

Introduction

Cereals are cultivated world-wide, wheat and rice with 1100 million ton/year (equivalent to the 56.7% of the total cereal yield) are of considerable importance for food production (FAO, 1993). In Europe the yield is 132 and 2.3 million ton/year for wheat and rice respectively. Italy with 1.2 million ton is the main rice producer in Europe. Only in the last ten years this crop began to be of importance in Portugal and Spain.

In normal agronomical practice rice is cultivated almost exclusively in paddy fields from sowing to grain filling. When rice seeds are sown in hypoxic also in anaerobic environment they have the peculiar characteristic to set-up exclusively by coleoptile elongation in order to reach the water surface and to enter in contact with the atmosphere oxygen, allowing a subsequent leaves and root growth.

The other cereals that sometime could experienced hypoxia after sowing, due essentially to strong rain with consequent flooding of soil, are not able to elongate their coleoptiles in absence of oxygen and the consequence is the failure of seedlings that cause a lost of yield.

Rice is therefore very interesting as a plant model to studies how plants can escape soil anaerobiosis, through coleoptile elongation.

It is well known, from the begin of auxin story in this century (Went and Thimann, 1937), that cereal coleoptile elongate on exogenous application of auxins.

Furthermore, phytohormones are know to have a role in the seed processes either the development and the germination (i.e.: Torti et al., 1986; Hilhorst and Karsen, 1992). The present paper addresses the question, by comparison between anoxia tolerant, rice, and intolerant, wheat, cereal species, about the possible involvement of abscisic acid and indoleacetic acid in the control of germination and coleoptile elongation in unfavourable anaerobic condition.

Materials and Methods

Plant material

Dehulled seeds of rice, japonica ecotype (*Oryza sativa* L. cv. Arborio, Ente Risi, Mortara, Italy) and of spring wheat (*Triticum aestivum* cv. Centauro, Istituto Sperimentale Cerealicoltura, Sant’Angelo Lodigiano, Italy) were used. The seeds were surface sterilised for 20 min with commercial diluted bleach (1:10, v/v) in flask. To favour seeds wetting, few drops of Tween 20 were added, and few minutes of reduced pressure was imposed to the flask. Seeds were than rinsed with several changes of sterile distilled water. Germination was carried out in dark at 27 °C in Petri dishes, on Whatman 3MM paper wetted and covered by 2–3 mm of water. Petri dishes were closed in AtmosBag (Aldrich Chemical, USA) in which aerobic or anoxia condition were imposed by air or nitrogen streams after filter sterilising and wetted by fluxing on water. Anoxia condition was obtained in 60 min and checked by the indicator of GasPack anaerobic system (BBL, USA).

Determination of free and conjugated IAA and ABA

Dry seeds were ground to a fine powder to pass 50 mesh in a Janke Kunkel mill. Coleoptile, root, and endosperm samples, from germinating seedlings, were ground in a mortar. The extractions were carried out with a mixture of 94% methanol : 5% water : 1% acetic acid, for free IAA and ABA. Residues were hydrolysed in 1 N and 7 N NaOH for analysis of conjugated portions of growth substances (Bialek and Cohen, 1992). Other samples of dry seed powder were directly hydrolysed in 7 N NaOH for total IAA and ABA determination. Methanol extracts and hydrolysed solutions were treated for the growth substances purification with ethylacetate partition and Sep-Pak C18 cartridges (Waters Ass. USA). The residues dissolved in methanol were analysed by HPLC with suitable column and solvents, IAA and ABA were quantified with fluorimetric and spectrophotometric detectors respectively. The putative phytohormone fractions were collected and tested for their nature by ELISA tests with monoclonal antibodies (Phytoscience, France). Lots between 20 to 40 seeds or seedling parts were used for each analysis that were repeated three time.

Results

Rice cv. Arborio and wheat cv. Centauro dry seeds contain IAA either in free or in conjugated forms, esters hydrolysable at room temperature in 1N sodium hydroxide, and total bound IAA (bound to macromolecules: proteins, carbohydrates) hydrolysable in boiling 7N sodium hydroxide. The free fraction is a minority part in both species (Table 1).

ABA is present in Arborio rice and Centauro wheat dry seeds. Also ABA is predominantly stored in the conjugated forms hydrolysable in 7N sodium hydroxide. Of particular interest seems the very low presence (trace) of the free ABA in Arborio rice dry seeds; on the contrary it is present in conspicuous amount in the wheat seeds (Table 1).

Table 1. Indoleacetic acid and abscisic acid content in rice and wheat dry seeds, extractable with methanol (free), 1N NaOH (ester bound), and 7N NaOH (total bound) (mg/g ± SE)

	Rice	Wheat
	µg/g of dry seed	
ABA free	0.005±0.002	0.047±0.012
ABA ester bound	0.022±0.006	0.035±0.008
ABA total bound	3.916±1.024	1.650±0.365
IAA free	0.561±0.081	0.161±0.046
IAA ester bound	0.017±0.010	0.056±0.020
IAA total bound	2.313±0.184	5.867±0.985

What happen to the phytohormones during the germination?

During rice germination an increase in the free IAA in the organs of seedling gives indication that a hydrolysis of conjugated IAA forms occurs, faster in air and at lower rate in anoxia (Table 2).

Table 2. Free IAA and free ABA in organs of rice seedling and in culture medium after six days of germination (ng/seedling ± SE)

	IAA ng / seedling		ABA ng / seedling	
	rice air	rice anoxia	rice air	rice anoxia
Coleoptile	1.14±0.35	0.09±0.02	4.82±0.83	1.83±0.12
Endosperm	5.65±0.88	15.93±2.05	8.21±1.12	3.07±0.71
Roots	0.64±0.12	*	6.92±1.37	*
Medium	0.45±0.06	0.35±0.08	2.15±0.80	23.56±2.65

* in anoxia germinated rice seedlings root is always absent

The distribution among seedling parts of free auxin after six days of germination shows that IAA is localised mainly in endosperm, with significantly difference between air and anoxia growth. The quantity released in the medium is not significantly different between the two condition considered. Also free ABA is present in seedling organs. Air grown seedlings have 2.6 time the quantity of ABA measured in anoxia germinated seedlings (Table 2). On the other hand, the ABA quantity released in the medium, already measurable in air growing conditions, become the predominant quantity of free ABA produced in anoxia condition. In six-day-old seedlings the total abscisic acid amount to 22.10 and 28.46 ng/seedling in air or anoxia grown respectively. Of them 9.7% in air and 82.7% in anoxia are released in the culture media (Table 2).

An evident ABA faster increase and release in the media is observable not only on anoxia germinated seeds but also on air germinated seedlings when those are subsequently subjected to anoxia (Fig. 1), indicating that the free ABA release in the medium is induced in rice as response to anoxia stress. The free ABA in medium is surely originated from the release of conjugated stored ABA but also the free ABA present in air growth coleoptile is depleted in the medium after 1 day of anaerobic treatment (Fig. 1).

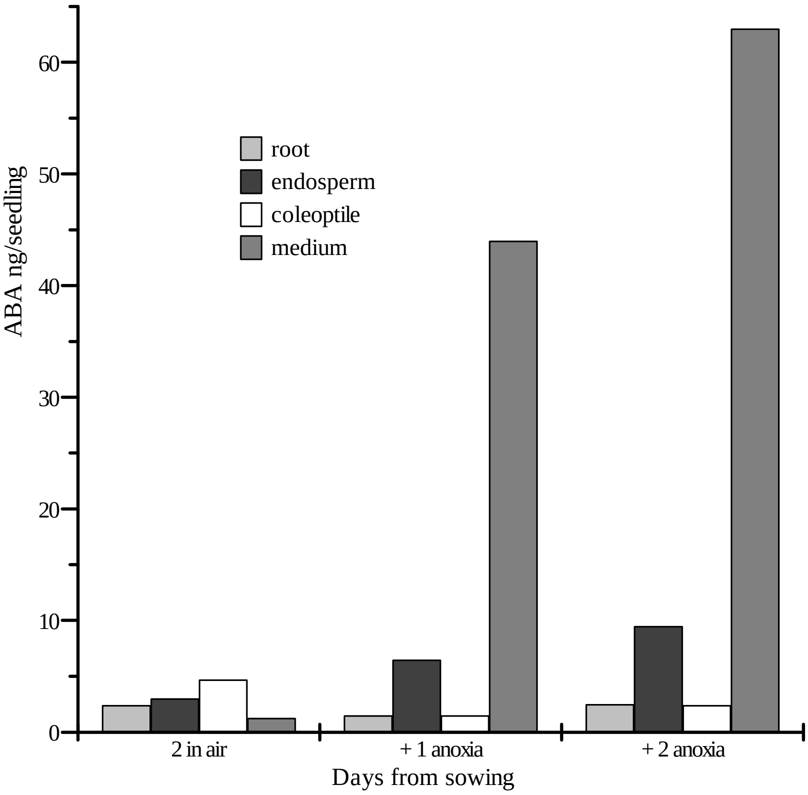

Fig. 1. Effect of anaerobiosis treatment on the ABA content and release from air germinated rice seedlings

Wheat does not germinate in anaerobiosis, so it was germinate in air and than transferred to anoxia. The elongation of coleoptile and root is totally blocked by anoxia. To check the vitality, after 2 days of anaerobiosis seedlings are transferred again to aerobic conditions. The seedlings are not died because the coleoptile growth recover and in three days it reaches a length comparable to the measure of continuously air grown seedlings of the same age.

The endogenous free IAA level in wheat seedling follows the same pattern of the growth in term of coleoptile length. When seedlings are subjected to anoxia the IAA quantity in coleoptile stopped, after return to air IAA in coleoptile rise to normal level (Table 3).

Table 3. Indoleacetic acid content and distribution in organs of wheat grown in air or germinated in air and then subjected to anaerobiosis treatment (ng/seedling ± SE)

	Indoleacetic acid ng/seedling			
	2 days in air	+2 days anoxia	+2 days anoxia +3 days air	6 days in air
coleoptile	39.93±4.28	41.81±5.16	63.28±12.43	79.93±8.38
endosperm	12.07±1.76	3.29±0.65	1.37± 0.07	7.07±1.03
roots	7.20±1.97	11.04±2.20	14.28± 2.85	12.07±3.45
medium	10.70±2.85	8.24±1.73	14.24± 1.65	14.64±5.02

Furthermore, the endogenous level of free ABA during wheat germination and growth immediately rises in 2 days air germinated seedling (Table 4). ABA increase is blocked when seedlings are subjected to anaerobiosis and it does not re-increase when seedlings are moved to air. Wheat seedlings either in air or in anaerobic conditions do not release free ABA in the media, on the contrary and of interest comparing to what observed when rice seeds are germinated, in anaerobiosis (Table 2).

Table 4. Abscisic acid distribution in organs of wheat seedlings grown in air or germinated in air and then subjected to anaerobiosis treatment (ng/seedling ± SE)

	Abscisic acid ng/seedling			
	2 days in air	+2 days anoxia	+2 days anoxia +3 days air	6 days in air
coleoptile	15.23±2.11	14.01±2.85	16.30±1.98	25.13±4.15
endosperm	5.00±1.56	4.14±0.83	0.96±0.03	4.18±1.09
roots	4.80±0.97	2.00±0.76	n. d.*	22.81±5.62
medium	0.80±0.09	0.57±0.12	n. d.*	0.98±0.35

* n. d. = not detectable, below the sensitive level of detector

Discussion

The analysis of endogenous IAA had shown that it was present either in air or in anoxia coleoptiles of intact rice seedling and increases with time, but there was no endogenous IAA increase when coleoptile was excised either from anoxia or air grown seedlings and cultured in the same environment conditions (Mapelli et al., 1988). This let to suppose that IAA was not synthesised in rice coleoptiles, and that IAA came from the endosperm, similarly to what suggested for oat (Kamisaka et al., 1989). The present paper confirms the possible endosperm origin of free IAA in growing seedling organs, of rice and wheat, as consequence of hydrolysis of IAA stored forms detected at very consistent level in dry seeds (Table 1). Few it is known about the nature of phytohormone conjugated forms stored in dry cereal seeds. The presence of IAA conjugated to proteins in storage reserve of seed endosperms is well documented for maize (Torti et al., 1986; Leverone et al., 1991) as well as for pea (Bialek and Cohen, 1992).

Abscisic acid is the only growth regulator, exogenously applied, that had shown the capacity to inhibit either air or anoxia growth of rice coleoptile (Horton, 1991; Mapelli et al., 1993). Also ABA is stored in dry seeds in conjugated forms mainly (Table 1), and during germination it is transported in free form to the developing seedling organs, either for rice (Table 2) and wheat (Table 4). It is therefore possible to think that the endogenous free ABA could be one of the causes that determine the different coleoptile length in air and in anoxia. Rice germinated in anoxia escapes the negative ABA effect on coleoptile elongation by a faster ABA release in the medium. The abscisic acid release in the medium is observable not only for anoxia germinated rice seeds but also for air germinated seedlings when those are subsequently subjected to anoxia, indicating the release as typical response to anoxia stress (Fig. 1). This is true and specific only for rice. Two-day-old wheat seedlings subjected to anaerobiosis maintain the original ABA quantity in each organs without any release in the medium (Table 4). Of course it was important to check if the released substances was true ABA. Different GC and HPLC chromatographic system analyses of substances recovered in the medium of anoxia seedling growth, UV spectra characteristics and the positive, and quantitative, response to ELISA assay against ABA monoclonal antibodies, give confirmation that the released compound is ABA really.

From present data on rice and wheat the answer to the involvement of auxin in the coleoptile growth is confirmed for air growing conditions. No further evidences are reported for its involvement in anaerobic growth. In anoxia endogenous auxin remains in fact at low level in rice, or at stable level in wheat. The presence of auxin binding activities and activation of steps reputed to be implicated in auxin signal perception has been reported for rice (Zaina et al., 1989, 1991), wheat (Zazimalova et al., 1985), and maize (Ray et al., 1977; Napier and Venis, 1990) seedlings grown in aerobic condition.

In rice, auxin binding activities, present in air, are absent or inactivated after two hours of nitrogen flux or when seeds are germinated in anoxia (Zaina et al., 1989). No data are reported at the moment about auxin binding activities in other cereals grown in anoxia environment.

If the questions is addressed to the ABA involvement in cereal coleoptile growth, the answer could be affirmative both for air and anoxia. Endogenous, from present data, or exogenous applied (Horton, 1991; Mapelli et al., 1993) ABA contributes to a reduced elongation rate and, it could be involved in the coleoptile opening, a senescence process. The rapid release in the medium of anoxia growing rice seedlings is needed to avoid the negative ABA action.

Abscisic acid and indoleacetic acid could be considered antagonistics in the control of cereal coleoptile growth in air. They are present in dry seeds (in free and conjugated forms), so it is not necessary their new biosynthesis during germination because it is sufficient their mobilisation from stored seed reserve. Furthermore, IAA and ABA could not be the only regulator substances involved in the anoxia elongation of rice coleoptile. In anoxia grown rice coleoptiles low ferulic acid content than air grown has been determined (Tan et al., 1991). Ferulic acid is involved in creating bridges that induced the rigidity of cell to cell structure. The relationship between ABA and ferulic acid or other phenolic compounds in the determination of lettuce hypocotyl elongation has been reported (Li et al., 1993). The reduction of endogenous ABA, here observed naturally occurring only in anoxia growth of rice, may drive to a higher cell wall and membrane extensibility through the alteration of the substances that determine the cell wall structure.

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