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I am going to talk about upland rice and aerenchyma. Let me explain.

Rice is a semi-aquatic species that grows well in water-saturated soils.
Maize and other cereals grows well in the opposite ecologies with well-drained soils.

	Rice	Maize
Flooded soil		
Drained soil		

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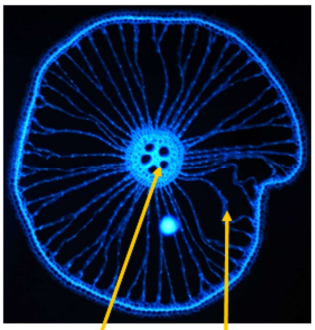
Rice is a very special cereal that grows well in water-saturated soils and not so well in drained soils. All other cereals such as maize or wheat have the opposite adaptation and dislike water saturation.
Thus, it's a perfect complement.

Why trying to improve upland rice yield potential?

- Millions of poor farmers grow 12 million ha of upland rice, certainly for good reasons
- Upland rice potential remains far from lowland potential and not competitive in market-oriented production, as in Brazil.
- Rice paddy fields emit methane. On drained, never flooded land, upland rice with improved yields would help reduce methane emissions.

Why targeting aerenchyma?



IR64 Root cross-section

- Wageningen and Yangzhou Universities in a project named 'Growing rice as wheat' showed that at flowering stage the upland variety APO had less aerenchyma than the lowland variety IR 64. Wheat does not produce aerenchyma.
- More aerenchyma associates with reduced radial transfer of water in the root.
- Rice lines without aerenchyma could possibly be created through CRISPR gene edition that would facilitate testing this new trait.

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So why try to improve the yield of upland rice?
. Because millions of poor farmers grow upland rice, and they have good reasons for doing so.
. Because the potential yield of upland rice is only 5 t/ha compared to 10 t/ha for lowland rice, which is not competitive in market-oriented production.
. Because flooded rice fields emit methane. On drained and never flooded land, high yielding upland rice could help reduce methane emissions.
And why target aerenchyma?
Aerenchyma are empty spaces in the parenchyma tissue of roots, stems and leaves. They allow gaseous diffusion of oxygen, methane, carbon dioxide and water vapour in aquatic plants.
In this cross section, we see an extreme case with no cells left in the cortex, only a few membranes remaining.
. During a Wageningen and Yangzhou Universities project named 'Growing rice as wheat', it was shown that the upland variety APO had less aerenchyma than the lowland variety IR 64 when observed at flowering time. The wheat varieties did not produce any aerenchyma. Under aerobic conditions, this is a crucial difference between the two crops. Rice has aerenchyma while wheat does not.
. Several authors have shown that radial water transport in roots is reduced when aerenchyma are present.
. Recently, it has also been suggested that aerenchyma-free lines might be produced by CRISPR editing.

Suppressing aerenchyma in upland rice?

Hypotheses:

. Aerenchyma reduces water uptake and consequently plant transpiration
. Natural variability for % Aerenchyma could be a source for further improvement in upland rice
. Aerenchyma formation could be knock-down in CRISPR-edited lines

Experiments:

MATERIAL

Ten varieties	Origin	Sub-species	System
SAHEL 108	Senegal	Indica	Lowland
IR 64	Philippines	Indica	Lowland
PRIMAVERA	Brazil	Tropical Japonica	Upland
CIRAD 409	Colombia	Tropical Japonica	Upland
KINANDANG PATONG	Philippines	Tropical Japonica	Upland
IRAT 109	Ivory Coast	Tropical Japonica	Dual
UPL RI 7	Philippines	Indica	Upland
APO	Philippines	Indica	Upland
CHHOMRONG DAHN	Nepal	Temperate Japonica	Dual
KITAAKE	Japan	Temperate Japonica	Lowland

Fourteen CRISPR-edited lines for three genes regulating the aerenchyma formation LOL1, HRE1, and HMA2, issued from KITAAKE.

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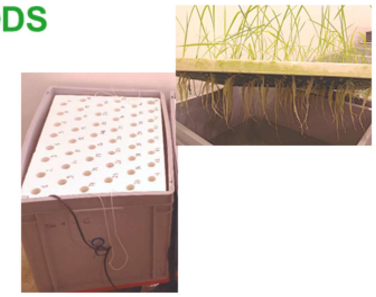
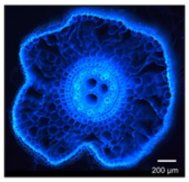
So we wondered about the effect of suppressing aerenchyma in rice.
We had three hypotheses that we started to test. 1) Aerenchyma reduce water uptake and transpiration of the plant. 2) Natural variability of the % of aerenchyma exists and can be used 3) CRISPR editing could create lines without aerenchyma
We took advantage of the existing variability and selected 10 varieties that were contrasted for three criteria: origin, subspecies and cropping system.
We also tested 14 CRISPR lines edited to knock out 3 regulatory genes.

METHODS



Eighty liter containers of hydroponic solution and 1.5 liter pots filled with horticultural substrate, all placed in tropical growth chambers.
Transpiration measured by weighing pots covered with plastic film.

Cross sections at 1, 2, 3 cm from the apex and 1cm from the base



% Aerenchyma = Lacuna area/cortex area in roots sampled at 15-25 days in cross sections imaged by epifluorescence microscopy.

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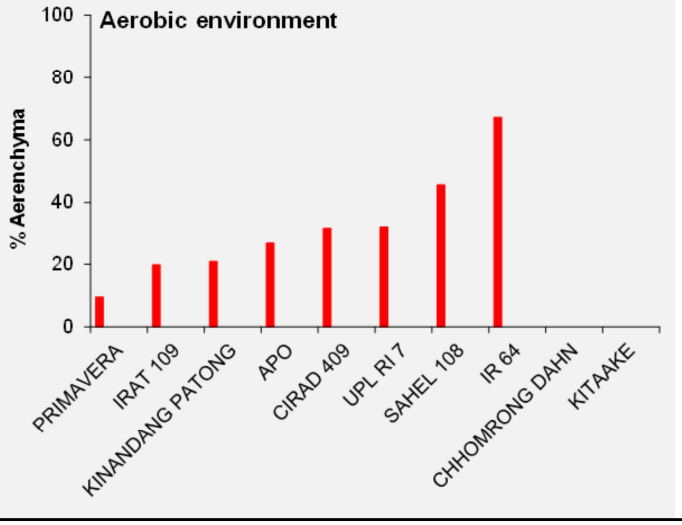
METHODS. Plants were grown in growth chambers either in large containers of solution or in pots filled with a substrate.
At the 5 to 6 leaves stage, roots were extracted and cross-sections were made in nodal roots at 1, 2 and 3 cm from the apex or 1 cm from the root base.
The sections were photographed with an epifluorescence microscope and the images analysed to obtain the percentage of aerenchyma, calculated as the ratio of the area of the lacuna to the area of the cortex.

RESULTS. % Aerenchyma in roots of 10 varieties grown in aerobic conditions

. In a solution, at 3 cm from the apex
. In a substrate, at 3cm from the apex and *1 cm from the root base*
. In the field, at 3cm from the apex and *1 cm from the root base* (Ouyang, 2020)



Clear sorting from upland to lowland varieties grown in a solution.

RESULTS. We were very excited by our first results on the % of aerenchyma at 3 cm from the apex of plants grown in solution. We observed a very clear gradient from the upland variety Primavera to the lowland variety IR 64.

RESULTS. % Aerenchyma in roots of 10 varieties grown in aerobic conditions

. In a solution, at 3 cm from the apex
. In a substrate, at 3cm from the apex and *1 cm from the root base*
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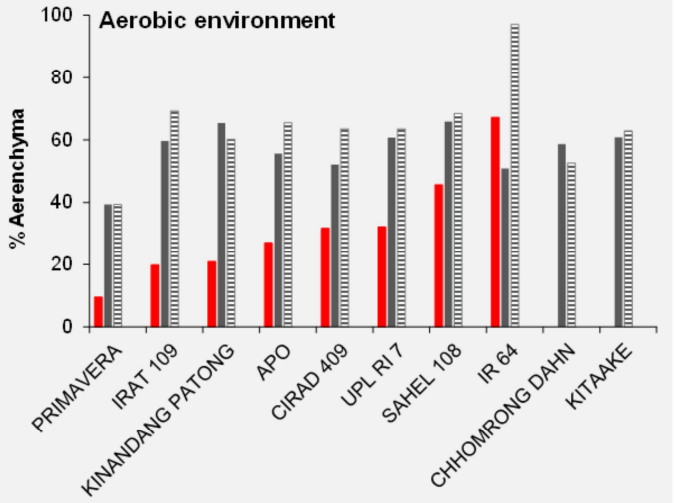

Clear sorting from upland to lowland varieties grown in a solution.

Only Primareva shows a clear difference in a substraste.

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However, for plants grown in substrate, this gradient was almost lost at both 3 cm from the apex and at 1 cm from the base, except for PRIMAVERA, the Brazilian upland variety, which still had less aerenchyma than the other varieties.

RESULTS. % Aerenchyma in roots of 10 varieties grown in aerobic conditions

. In a solution, at 3 cm from the apex
. In a substrate, at 3cm from the apex and *1 cm from the root base*
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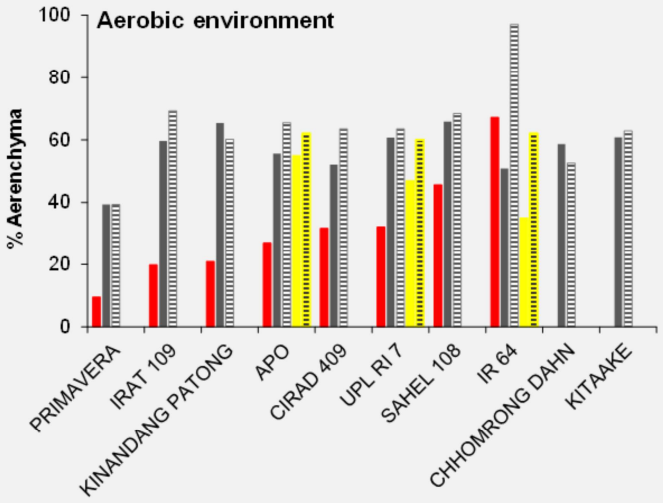

Clear sorting from upland to lowland varieties grown in a solution.

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Consistency with the previous results in the field

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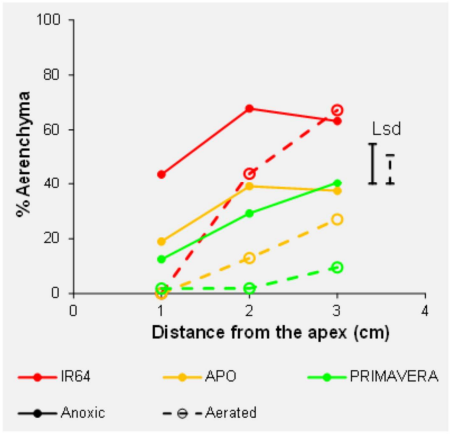
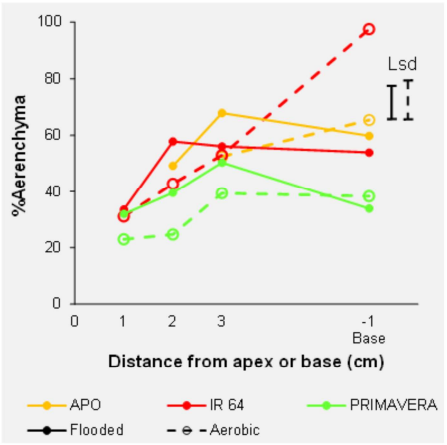
In addition, our results were consistent with the previous results from Wageningen and Yangzhou Universities for 3 common varieties.

% Aerenchyma increases strongly with distance to the root tip between 1 and 3 cm and only slightly beyond 3 cm to the root base.
Between 1 and 3 cm, % Aerenchyma increases more rapidly in anoxic than in aerobic conditions. Beyond 3 cm, % Aerenchyma was generally independent of irrigation.

In hydroponic solution.
%AA at 1, 2, and 3 cm from the apex



In horticultural substrate.
%AA at 1,2,and 3 cm from the apex and 1 cm from the root base



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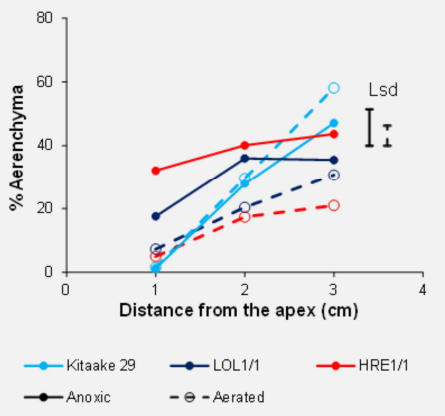
We clarified a technical point that was not so clear in the literature.
We observed (**left graph** with 3 varieties) that aerenchyma are formed in the first 2 to 3 cm from the apex and not beyond.
They are formed earlier in anoxic than in aerobic conditions, but beyond 3 cm from the apex the percentage of aerenchyma was generally similar in both anoxic and aerobic conditions (**right graph**).

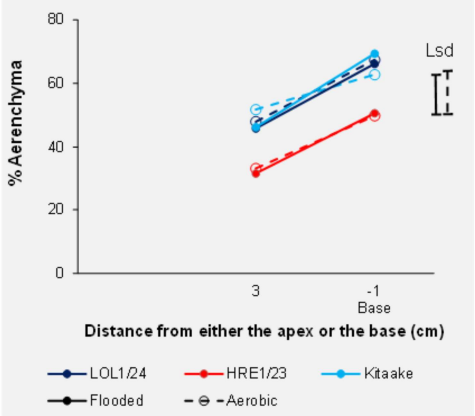
% Aerenchyma in 14 CRISPR-edited lines

. In solution, % Aerenchyma at 3 cm was lower in the 6 lines than in Kitaake, and lower in aerobic than in anoxic conditions.
. In the substrate, only one out of eight lines had less aerenchyma than Kitaake. Soil water content had no effect.

In hydroponic solution.
%AA at 1, 2, and 3 cm from the apex (left to right)



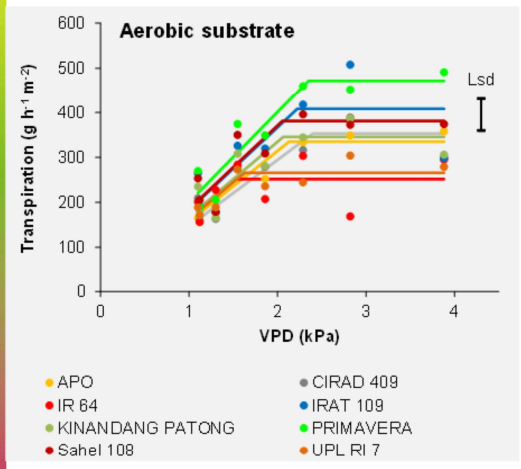
In horticultural substrate.
%AA at 3 cm from the apex and 1 cm for the root base (left to right)

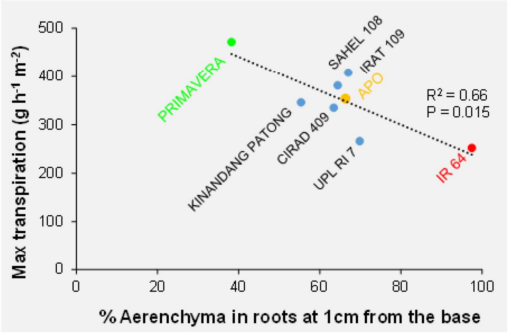


Here, our first results with the CRISPR-edited lines. We had two different sets of lines.
Left graph: In solution, 6 edited lines always had less aerenchyma than their parent KITAAKE at 1 to 3 cm from the apex.
Right graph: In the substrate, only one of 8 edited lines had less aerenchyma than KITAAKE. Still increasing beyond 3 cm in this experiment with these lines.

In the aerobic substrate, maximum transpiration was significantly higher in Primavera than in IR 64.

Maximum transpiration was negatively correlated with % Aerenchyma at 1cm from the root base.





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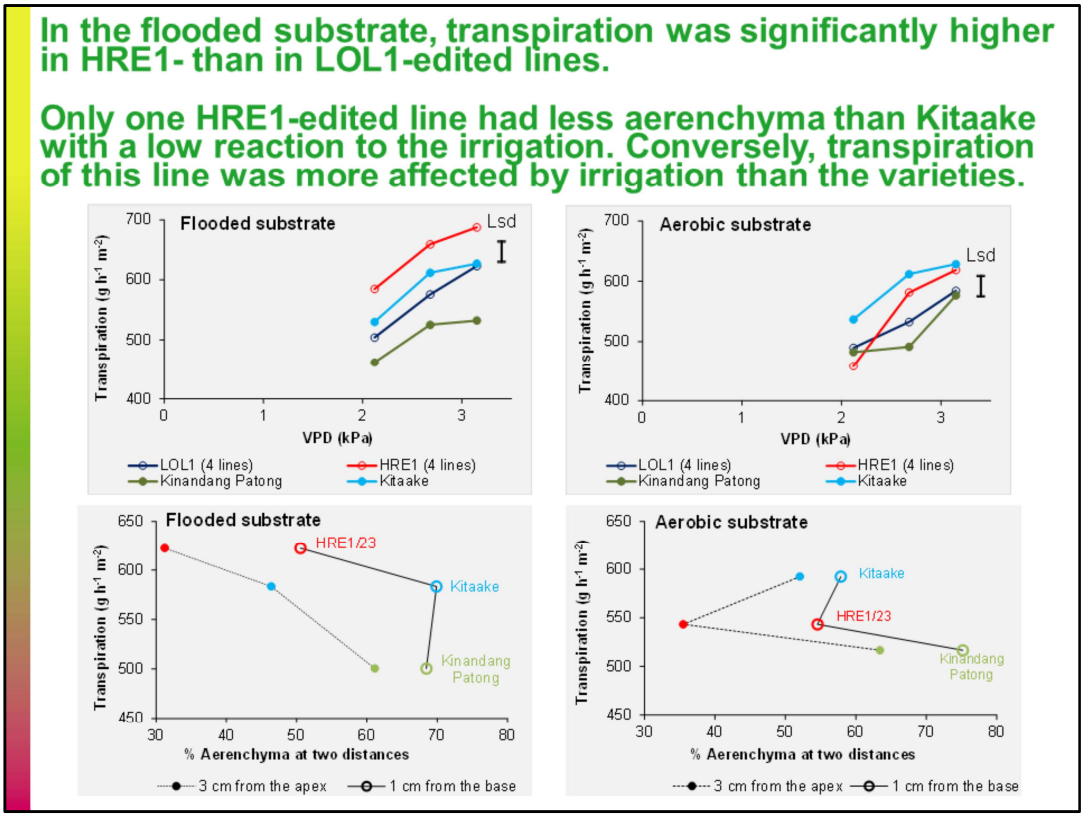
We measured the transpiration of 8 varieties in response to the evaporative demand (the VPD) and observed that the maximum transpiration in aerobic conditions was twice as high in PRIMAVERA than in IR 64. **(left graph**).
Right graph: This maximum transpiration was significantly correlated with the % of aerenchyma of the 8 varieties.



Finally (**top graphs**), the only edited-line with less aerenchyma than Kitaake transpired more than KITAAKE in the flooded substrate but less in the aerobic substrate. Consequently (**Bottom graphs**), the percentage of aerenchyma and the transpiration were correlated only in the flooded substrate.

Conclusions

- The Brazilian upland variety Primavera produced less aerenchyma than every other varieties.
- Negative correlation between plant transpiration and % Aerenchyma at 1 cm from the root base.

Perspectives

- Aerenchyma could be a good trait to improve upland rice varieties.
- Aerenchyma constitute a network in plants from leaf midrib to root tips. It would be logical that the size of this network varies proportionnaly in the roots and in the leaves. We will check this hypothesis. If true, it will be easier to determine % Aerenchyma in leaves than in roots. (Poster # 96).

We have two main conclusions:
1. The Brazilian upland variety PRIMAVERA has less aerenchyma than any other variety.
2. There is a negative correlation between plant transpiration and the percentage of aerenchyma.
Perspectives:
. Roots aerenchyma could be a good trait to improve upland varieties.
. But practically useless because it is too difficult to work with roots. However, there is a continuum of aerenchyma in plants from leaves to roots and we can expect that the variability of the % of aerenchyma will be similar in the different organs. We are now testing this hypothesis. See poster no. 96.

Thanks to the audience, to the co-authors who did the work with me and to CIRAD and Agropolis Fondation who funded the research and my participation in this event.