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Strategies for rice salinity tolerance in Mediterranean France

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Abstract. Salinity constraints for Rice cultivation in Camargue mainly concern short periods (5-10 days) during the first cropping month (from germination to 5th leaf stages). Because of unfavourable climatic conditions, it is impossible to directly undertake breeding lines field screening. So greenhouse tests have been performed in order to specifically screen for varietal salt tolerance according to the Rice critical stages in Camargue: germination (comparison of germination ratio in salted/unsalted conditions), sprouting (evolution of dry matter weight in function of increased salt concentration) and 5th leaf stage (transpiration and photosynthesis activities evolution in function of increased salt concentration). Another type of test attempt to consider the effect of cumulated constraints; the varietal behaviour when salted and cold environment give a good image of that really occurs in Camargue. Then, fields trials only concerns the better genotypes in order to confirm greenhouse tests characterisation.

Another way to improve salt tolerance try to profit by the presumed "seeds hardness" phenomenon. Using three seeds samples coming from cropping in very different situation of salt constraint (weakly, moderately or strongly salted conditions) and three type of conditions for germination (distilled water, 2.54 g/l NaCl and 10.2 g/l NaCl), it is possible to measure "the hardness seed effect". Whatever the salt concentration, the germination rates are so much higher so was the importance of salt constraint in the seed multiplication paddyfield.

Prospective for Rice salt tolerance improvement, based on the knowledge of salt adaptation cells physiological mechanism is discussed.

Key Words. Rice – Salt tolerance – Breeding – Germination-sprouting stage – Greenhouse tests – "Seed hardness" – Biotechnology.

Rice (*Oryza sativa* L.) is moderately sensitive to salt in the field as almost all the other crop species. However, rice is the only one crop which be cropped in these salty soils because it tolerates flooded conditions (a lot of Rice growing land are, for this reason, located on coastal areas); recharge of ground water , fresh water regular supplies and artificial drainage allow to push out salt and to avoid reconcentration by capillarity.

Camargue land include a large part of salty soils with an heterogeneous distribution depending on the geologic origin of the Rhône delta soils (river old courses, lagoon basins, ...). Fresh water being available by pumping in the Rhône river, the salt presence have not a direct influence on Rice growth (thought it prevents crop rotation possibility and its improving effects). In spite of this *a priori* favourable situation, salt problems can affect Rice cultivation in Camargue for two main reasons:

❑ because Camargue is located at the North limit of Rice cultivation extension, the seedling must be done during a short period (from April 20 to May 10) in order to have flowering time before August 15; after this date, cold conditions can induce important yield losses by panicular sterility. Generally, climatic conditions are not so good during the seedling time (Ice Saints period) with frequent cold temperatures and strong and dry northerly wind affecting the germination-sprouting stage quality:

- cold conditions delay the germination and increase the pathogens damages on the seeds and the plantlets;
- because Rice seeds are sowed on the ground, water movements bury the seeds preventing the germination;

- during sprouting stage, the same water movements uproot the plantlets and move them to the paddyfield edge. This type of damage is strengthened by the incidence *of Chironomus* larvae which cut the young roots.

The only method to save the Rice cropping is to rapidly drain the paddyfield in order to improve the anchorage of Rice plants. In these conditions, by the dry wind evaporation effects, salt goes up by capillarity and can cause important direct damages on germination quality or on the rice platelets survival.

❏ The Rhône river discharge is generally sufficient to have fresh water disponibility. Sometimes, and according to rainfalls or snowfalls degree in the mountains, discharge river can be low enough to allow the sea salty water filling the lower river course and salty water can be pumped for paddyfield flooding. This problem can occur whatever the crop stage. Fortunately unusual, the problem is rapidly solved by the way of the Rhône water (quantity and quality) survey system and a discharge regulation from water dams management.

Salinity constraint for Rice cultivation in Camargue, according the induced damages degree, mainly concerns short periods of stress (5-10 days) during the germination-sprouting stage but salt injury can be registered until the 5th leaf (beginning of tillering) stage. Some control strategies, the present leaflet aims to report the main results, have been undertaken in order to decrease the salt negative effects during the beginning of Rice cultivation. They concern the use of intrinsic or acquired varietal tolerance. Further strategies, elaborated from the salt tolerance physiological knowledge will be presented too.

I – Screening rice varieties for salt tolerance

Screening a lot of Rice lines for salt tolerance during the breeding process is difficult to carry out in the Camargue Rice fields because the wind effects cause an unavoidable seed mixture. On the other hand, transplanting is not efficient because it doesn't allow to test the genotypes sensitivity during the critical stage.

Greenhouse tests are not wholly satisfactory. They effectively allow to test a large scale of genotypes but they only consider the salt constraint when, in the field, other biotic or abiotic constraints can modify the tolerance degree (cumulated constraints effects).

So, in our experimental design, greenhouse tests are undertaken to have a first genotype sort ,and then, the best lines are laid out in the field, on 50 m^2 plots minimum, in order to validate the results obtained under controlled conditions.

Three types of salt tolerance tests are done according to the critical Rice stages in Camargue: germination, sprouting and plantlet (till 5th leaf-beginning of tillering stage).

1. Determining Rice salt tolerance at germination stage

This test aims to study the salt direct influence on germination for a given variety sample. For this purpose, a simple germination test is undertaken in standard conditions (Petri box, 30°C temperature). For a varietal sample, a comparison between the germination rates, got in distilled water and in a 2 g/l NaCl solution allow to determine salt tolerance (observations are done 14 days after sowing).

Figure 1 reports the results recorded from 22 varieties. The datas show that the germination ability of 2 varieties, Thaïbonnet and Koral, is drastically affected by salt presence even it is stated that Rice can admit 2 g/l NaCl concentration. For almost all the other varieties, the germination ratio decrease, but no significantly (except for Barragia variety), with salt presence.

Field observations confirm that the seedling survival of Koral and Thaïbonnet varieties is generally poor when cropped in salty soils (and, by extension, in soils known to be of a difficult cultivation).

This type of test is very simple to undertake. It is unefficient to detect the better salt tolerant varieties but it allows to characterise (and eventually eliminate) the worst.

2. Determining salt tolerance at sprouting stage

The objective of the test is to determine the incidence of a period of salt presence occurring during the sprouting stage after a germination under standard conditions. In this test, race seeds are set germinating in distilled water, with 30°C temperature, during 5 to 10 days. Then, the sample is divided, a part going on sprouting in distilled water when others parts are set in variable concentrations NaCl solutions (1.27 g/l to 10.2 g/l). After 20 days, the dry matter plantlets weight of the reference (distilled water) and the treatments (salty solutions) are compared. The produced dry matter decreases when the salt concentration increases, but the response curves depend on each variety. So, it is possible to rank them in relation to the percentage of dry matter reduction for each salt concentration.

Table I reports the qualitative classification (5 classes from the best to the worst) done from 29 tested varieties. These datas are demonstrative of a salt tolerance scale among varieties adapted to mediterranean France conditions. No varieties are classified in the very good salt tolerance group when only one variety is included in the very bad salt tolerance group. Four varieties, SP2, CNA 6159, Lord and K 1952 correctly behave in presence of salt. We can point out that the Koral variety, which present a very bad salt tolerance at germination stage is rather well classified according to a salt constraint occurring at sprouting stage; this result show the physiologic complexity of salt tolerance physiologic mechanisms and confirm the necessity to have a global evaluation of tolerance by the way of field tests.

This type of test is overall useful to characterise the working collection varieties for salt tolerance ability during the sprouting stage and then to perform the genitors choice before hybridization.

3. Determining salt tolerance at 5th leaf stage

A third type of test aims to evaluate salt tolerance at 5th leaf stage through the evolution of 2 physiologic parameters, photosynthesis and transpiration, for increasing concentration of salt. For this purpose, Rice seeds are sowed in pots filled of sand. Till the sprouting time, sand is soaked with distilled water and then with a nutritional solution (HOAGLAND type). After 20 days cultivation, the Rice plants are set in a special cultivation room allowing gas variations measures; from water vapour and carbonic gas variations in function of diverse salt concentration solution supplied to the roots, formulas allow to evaluate photosynthesis and transpiration rates. These datas are compared to the reference value and curves are established by the way of photosynthesis or transpiration degree (expressed in % of the reference) in function of salt concentration.

Figures 2, 3 and 4 show, for increasing salt concentration, the evolution of photosynthesis degree for 3 varieties: Cigalon, Ariete and CrodoGribe M. In spite of the photosynthesis degree with salt absence is very different for the three varieties (in mg $CO_2.dm^{-2}.h^{-1}$, 36.7 for Cigalon, 25.7 for Ariete, 17.0 for CrodoGribe M), the decrease of photosynthetic activity is similar, for the 3 varieties for increasing salt concentrations till 10.5 g/l NaCl (even if a low salt concentration seems to dope CrodoGribe M photosynthetic activity). The photosynthetic activity stops for 14 g/l and 15 g/l NaCl concentration for, respectively, Cigalon and Ariete when it goes on even with 20 g/l NaCl concentration for CrodoGribe M.

Figures 5, 6 and 7 report, for increasing salt concentration, the evolution of transpiration degree for the same 3 varieties. Under salt absence, the transpiration (in g $H_2O.dm^{-2}.h^{-1}$) degree is similar for Ariete and Cigalon (2.6 and 2.9 respectively), and weaker for CrodoGribe M (1.8). The salt response curves are different for the 3 varieties:

❏ Cigalon very quickly reacts, its transpiration decreasing from 80% of the reference for 4 g/l NaCl concentration till 10% for 6g Nacl concentration. Then, the transpiration degree remains stable;

- ❏ the Ariete transpiration degree gradually decrease untill becoming absent for 14 g/l NaCl concentration;
- ❏ the Crodogribe M response curve is almost linear.

The interpretation of this type of test is strictly depending on the question “What is the better varietal behaviour, according to the evolution of the two physiologic parameters considered, in case of salt presence?”. A plant which its transpiration decreases in presence of salt pumps less salty water and so, the time to reach the critic salt rate in the vegetal cell will be longer. On the other hand, a plant which maintains a certain photosynthesis degree in case of salt presence can goes on working without using the carbohydrate reserves of the leaves and stems (typical discoloration of the leaves as a symptom of salt injury).

So, the better compromise is to get a Rice variety which, in salt presence, can reduce its transpiration and, at the same time, can maintain a certain photosynthetic activity.

Table 2 reports, for the 3 varieties considered, the part of photosynthetic and transpiration activities in presence of 4 g/l (frequent in Camargue) and 6 g/l (fortunately unusual in Camargue) Nacl concentration according to the figures 1 to 6.

Table 2. % of photosynthetic and transpiration activities for 3 varieties in presence of 2 concentrations of salt with regard to a unsalted conditions reference

Variety	% of photosynthetic activity/reference		% of transpiration activity/reference	
	4 g/l Nacl	6 g/l Nacl	4 g/l NaCl	6g/l Nacl
Cigalon	90	85	80	10
Ariete	90	85	90	70
CrodoGribe M	90	80	70	60

For 4 g/l NaCl, the 3 varieties show the same behaviour when, for 6 g/l NaCl, Cigalon which significatively decrease its transpiration with a correct photosynthetic activity degree, show the better salt tolerance according to the better compromise we are looking for.

This type of test is very interesting to screen the released varieties under Camargue conditions where salty conditions duration is more or less short. Maybe a transpiration strong reduction and a certain photosynthetic activity are not so efficient to solve salinity problems of different nature such as permanent salinity presence or salinity occurring later during the Rice cycle.

4. An attempt to screen for cumulated constraints

Even if the seeds used in Camargue are of an excellent quality, a large proportion of seeds are lost during the germination sprouting stage. Besides the pathogens damages (often undirect of slow germination sprouting stage), cold, salt presence and oxygen lack seem to be the main factors explaining the particular low germination - sprouting rates registered.

An analysis of the cinetic of germination under simple (cold or salt) and cumulated (cold and salt) constraints was carried out for 4 varieties: Gandruk (nepalese variety with a very good cold tolerance) and three varieties, Indio, Lido and Thaïbonnet cultivated in Camargue. Table 3 reports, for the 4 varieties, the germination ratio recorded after 14 days and the numbers of days to first seed germination under salt (2 g/l NaCl and 30°C) or cold constraints (13.5° C temperature that correspond to mean temperature recorded in Camargue during the sowing time) and under cold and salt constraints (13.5° C and 2 g/l NaCl).

Table 3. Germination ratio and days to first germination under salty (2 g/l NaCl), cold (13.5°C) and cumulated conditions for 4 varieties

Variety	Germination ratio (after 14 days)			Days to first germination		
	Salt	Cold	Salt + Cold	Salt	Cold	Salt + Cold
Gandruk	100	90	88	3	4	3
Indio	100	79	38	5	7	8
Lido	100	74	28	3	4	8
Thaïbonnet	19	33	5	7	7	13

According to the four varieties, the effect of cold or salty conditions seems more or less comparable on both germination rates (even if cold seems to lower Lido and Indio germination rates and, in opposite, improve Thaïbonnet germination ratio) and days to first germination.

The cumulated presence of salt and cold don't affect Gandruk variety when Lido, Indio and overall Thaïbonnet are very sensitive: the germination ratio significatively decrease and the time to first germination is delayed.

This type of test gives a better image of that really occurs in Camargue and, for this reason, it is well adapted for varietal screening before registration proposals.

5. Discussion

Under Camargue conditions, we have to consider rather cumulated constraints than only salt constraint. In this framework, it is obvious that the breeding program aims to get a varietal behaviour such as Gandruk. Because of excessive late flowering under Camargue conditions and others undesirable characters according to our breeding goals, the use of foreign varieties like Gandruk in our crosses program is often embarrassing because of the tardivity of the progenies. On the other hand, the genetic variability for both cold and salt tolerance is poor in our working collection. The lack of suitable genitors is now the main limit to genetically progress for germination-sprouting stage improvement in spite of we dispose of efficient greenhouse tests for screening.

II – Acquiring salt tolerance by effect of precedent cropping

The agricultural popular wisdom get use to say that, when plants grow in a environment with a particular constraint, the descendants acquire a certain tolerance degree to this constraint and a better behaviour in standard conditions ("seeds hardness"). The production of rootstocks in difficult conditions to have a better vigour of the grafted plants is a well known concrete agricultural application of this oral tradition.

In order to argue about the "seed hardness" innuendo, some tests have been carried out to analyse the eventual effect of the salty/unsalted seeds multiplication conditions on their germination ability. Three type of seeds multiplication (Ariete variety) conditions are considered: weakly salty (S1), moderately salty (S2) and strongly salty (S3) conditions. In this last type, seeds multiplication was only possible by continual recharge of fresh water all along the Rice cycle. For seeds coming from each site, germination rates were evaluated in 3 conditions: distilled water, 2.54 g/l NaCl solution (concentration often occurring in Camargue on sowing time during the poor rainy winter and spring) and 10 g/l NaCl solution. The test was managed under 30°C temperature and darkness during the 4 first days and then under ambient temperature (more or less 20°C) and lightness. Observations were done 4, 5, 7 and 10 (green plantlets stage) days after sowing. Figures 9 and 10 report the seeds germination comparisons

1. For 2.7 g/l Nacl concentration by comparison with distilled water (Figure 9)

The results show , whatever the salt concentration, is differences between the germination rates in function of seeds origin (significatively different between S1-S2 and S3). The number of germinated seeds is so much higher so was the importance of salt constraint in the seeds multiplication paddyfields. The “hardness seed effect”, without presuming about the involved physiologic mechanisms, is measurable.

2. For 10 g/l NaCl concentration by comparison with distilled water (Figure 10)

Even if the high NaCl concentration was detrimental on plantlet survival 10 days after sowing, the results confirm the improvement of germination ability proportionally to the increased salt degree in the seeds multiplication paddyfields.

During the germination stage in salty water and after seed soaking, the salt slowly goes into the cells and gradually modify the cell internal osmotic pression. The seeds coming from a crop in salty conditions seems capable of “an osmotic adjustment” allowing a better salt tolerance. Maybe according the antiseptic role of salt, the seeds acquire at the same time a higher germination ability than seeds cropped in unsalted environment even in standard conditions Fields tests undertaken in Camargue from the same seeds samples confirm the greenhouse results.

This work first shows the interest to produce Rice seeds in the production land in order for the seeds to acquire a certain adaptation to the environmental constraints (including salt) and then better behave during the germination sprouting stage. Even if the better germination rates, in the considered test, are obtained with seeds cropped in a strongly salted environment, such a seed production is not economically suitable because of cropping difficulties and low yields. In the opposite, a way to improve the particular low germination sprouting rates registered in Camargue would be to avoid last generation seed multiplication cropping on paddyfields known to be free of constraints. In reality, because seeds production is a good business for Rice farmers, the better paddyfields are dedicated to.

For further research, it would be interesting to know if this acquired salt tolerance during the germination-sprouting stage continue to be effective all along the Rice cycle (particularly during flowering time)

Conclusion: prospective for rice salt tolerance improvement

The prospective for salt Rice salt tolerance improvement are based on the knowledge of salt adaptation cells physiological mechanisms that a schema is reported on Figure 11. The cell life depend on an osmotic equilibrium, based on ion exchanges, between internal and external pression. In case of NaCl presence, the cell can be destroyed by explosion or implosion due to the pression difference because of the ionic equilibrium is broken down. The cell seems capable to perform this ionic exchange in NaCl presence but wasting a lot of energy. It is now known that a 70 K Dalton protein, located in the tonoplast, is involved in the active ionic exchange; this protein allows ion transport, in case of Nacl presence, with a low energetic cost.

The presence of this protein was identified in tobacco (var Wisconsin 38) and so, it is possible to consider transfer by biotechnologies the gene determining this protein in cultivated Rice. This work can be better considered as the presence of a similar mechanism is probable in particular Rice varieties, called mangrove Rice (which are cropped on estuaries salty lands during the rivers flood). Rice being a model plant for biotechnologies application, and with the insurance this gene was already efficient in Rice, this work could be rapidly carry out with, on medium term, a drastic increase of salt tolerance in Rice. Such a strategy asks two complementary questions. “Is this protein activity compatible with economically suitable Rice yields in case of strong salt constraint?” and, if the response is affirmative, “what would be the maximum of salt degree constraint for which Rice yields remains economically suitable?”. The responses to this two questions will lead either to extend Rice cropping on strongly salty lands or to increase or regularise the yields (and/or to reduce the cultivation costs) in now cultivated Rice lands with an endemic salt constraint… An hard future work for the agronomic research teams!

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Figure 1. Salt influence on 22 varieties germination rates (under standard temperature conditions)

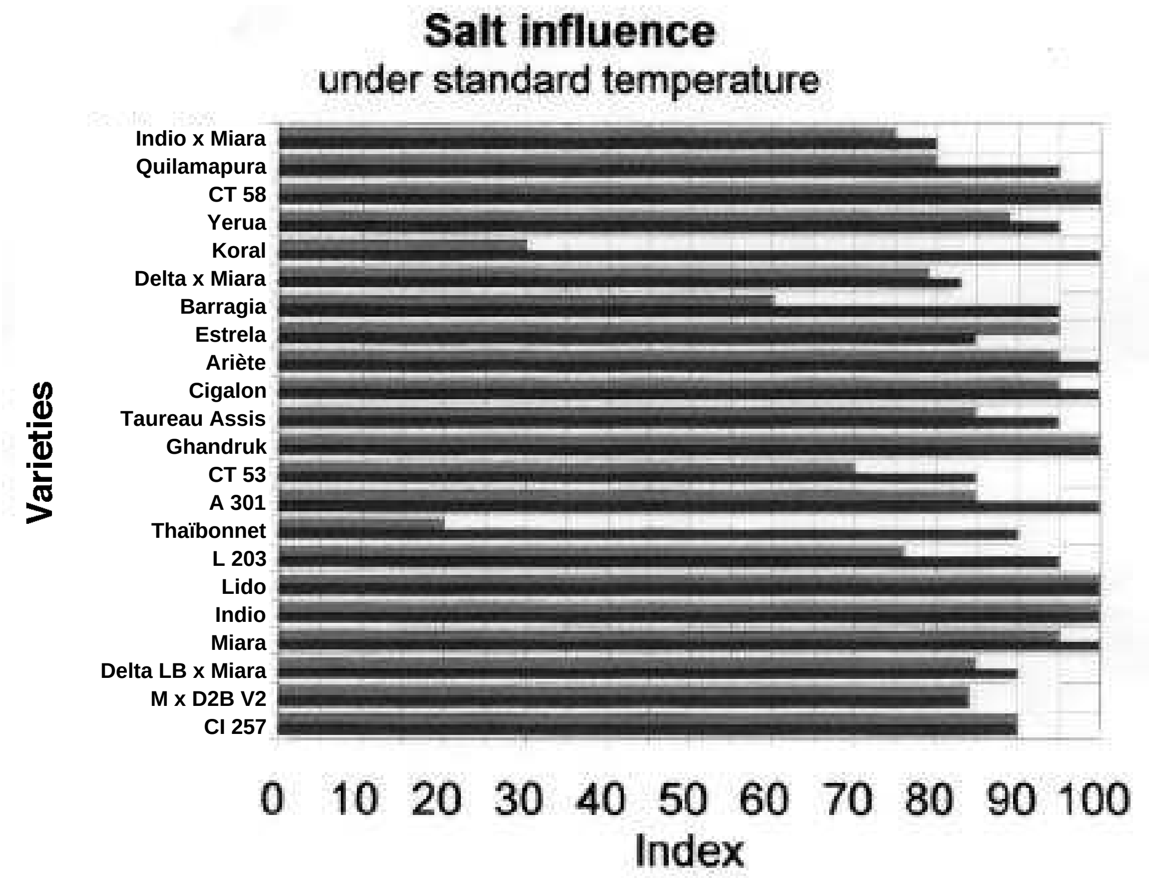


Tableau 1. 29 varieties ranking for dry matter production index after 20 days growing in nutritive solution with increased salt concentration

N°	Variétés testées	poids de 1000 Grains	classement des poids de semences, 1er le plus lourd	classement pour la résistance au sel à des concentrations exprimées en g/l de NaCl :						classement par catégories				
				0.5	0.8	1.5	2	4	Ms	TB	B	AB	M	TM
1	**Star**	33.3	10	23	19	3	12	28	17					
2	**Irat 112**	35.7	5	1	12	6	18	23	12					
3	**Italico-M**	29.2	20	28	18	18	15	8	17					
4	**San Andrea**	36.6	3	13	28	12	14	27	19					
5	**Lord**	28.8	22	6	4	10	3	26	10					
6	**Callenolet-E**	33.6	8	18	-	23	9	20	18					
7	**Catalirfroid-T**	27.6	25	9	-	7	25	29	14					
8	**Ariete**	29.5	17	11	29	15	22	11	18					
9	**Bahia**	34.0	7	12	22	1	23	19	15					
10	**Alpha**	29.3	18	27	3	19	10	25	17					
11	**Cropelta-G**	32.0	13	3	21	14	16	9	13					
12	**Irat 216**	24.0	27	26	24	18	4	7	14					
13	**Onda**	29.3	18	7	26	28	17	21	20					
14	**Ringo**	30.8	15	22	29	29	28	2	22					
15	**Cropelta-TJ**	29.2	20	20	15	20	21	12	18					
16	**SC2**	31.2	14	5	9	13	2	18	9					
17	**Rocca**	35.4	6	4	14	25	27	24	19					
18	**CNA 6159**	19.5	28	24	5	2	1	1	7					
19	**Koral**	30.1	16	15	7	17	11	6	11					
20	**Lido**	24.8	26	16	19	16	20	15	17					
21	**Crodogrilo-M**	40.2	2	2	29	24	19	16	18					
22	**Volano**	43.7	1	19	8	22	26	13	18					
23	**Sonia**	33.0	12	17	17	26	7	14	16					
24	**K 1952**	33.4	9	14	1	21	8	4	10					
25	**Cripto**	28.3	24	10	-	8	5	22	11					
26	**Cigalon**	28.4	23	25	6	5	29	3	14					
27	**CNA-4081**	23.1	27	8	21	4	6	17	12					
28	**Barragia**	33.1	11	21	20	27	13	10	18					
29	**Cropelta-D**	36.5	4	-	25	9	24	5	16					

Figure 2 to 7. Photosynthetic activity index for 3 varieties in relation to nutritive solution with increased salt concentration

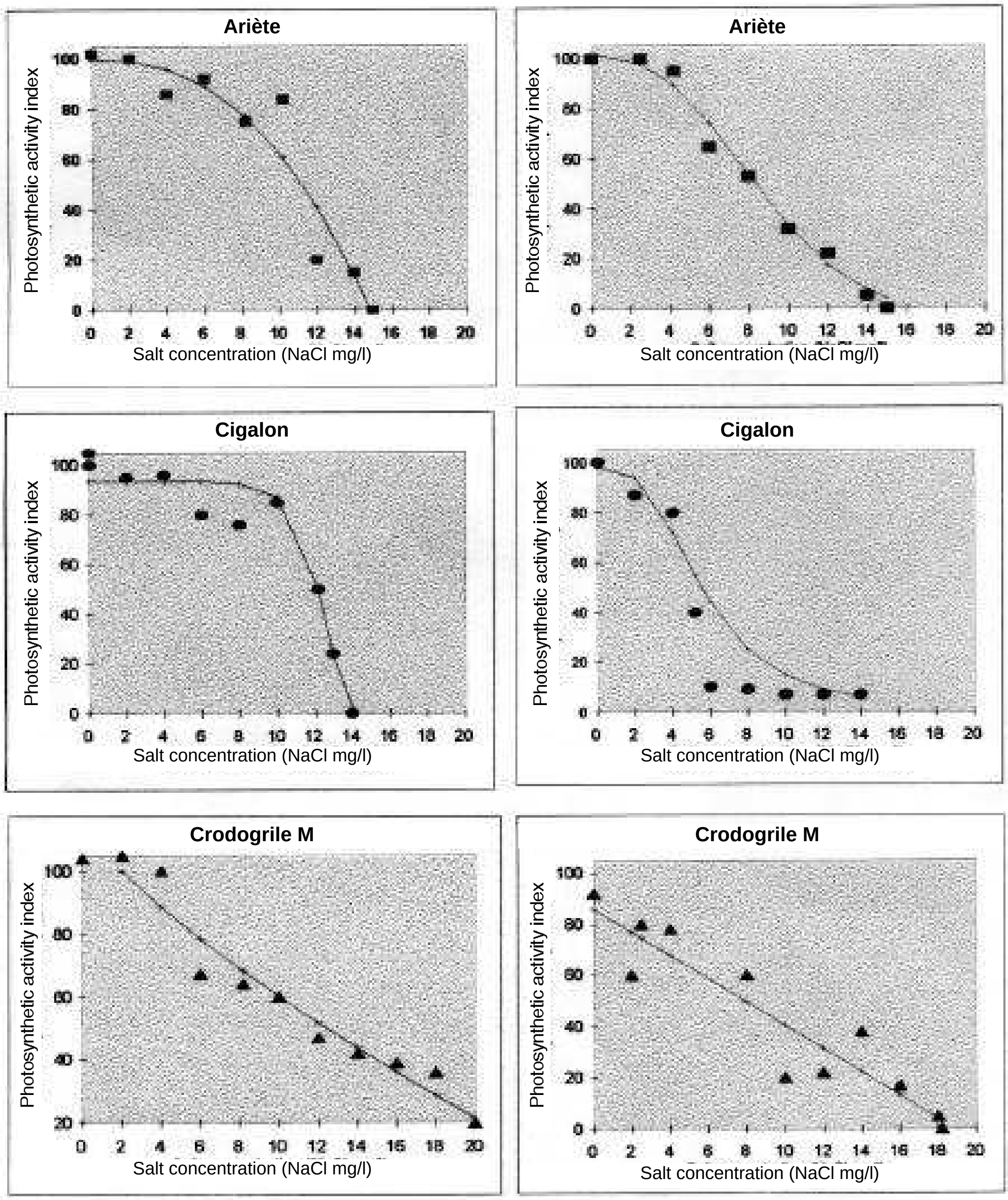
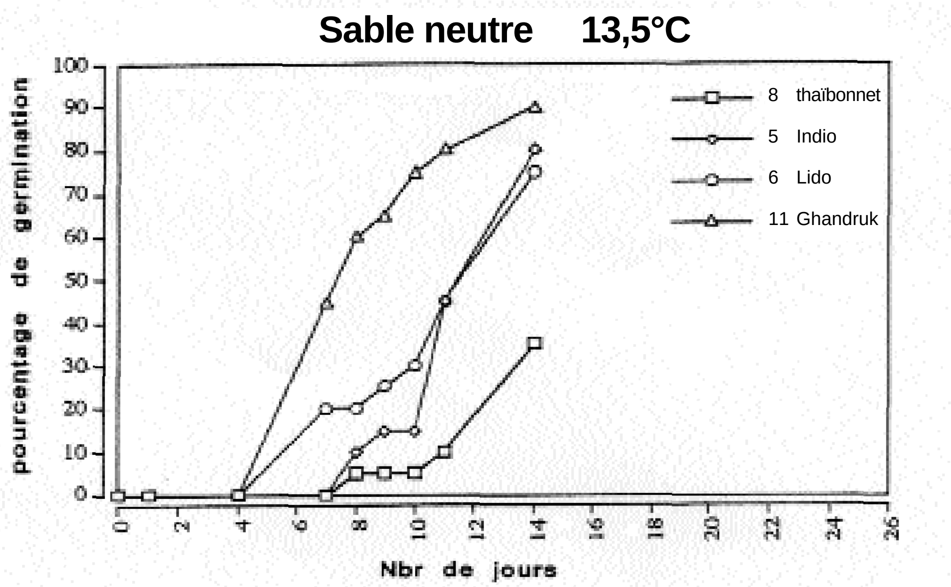


Figure 8. Germination cinetic of 4 varieties under simple (cold) or double (salt + cold) stresses



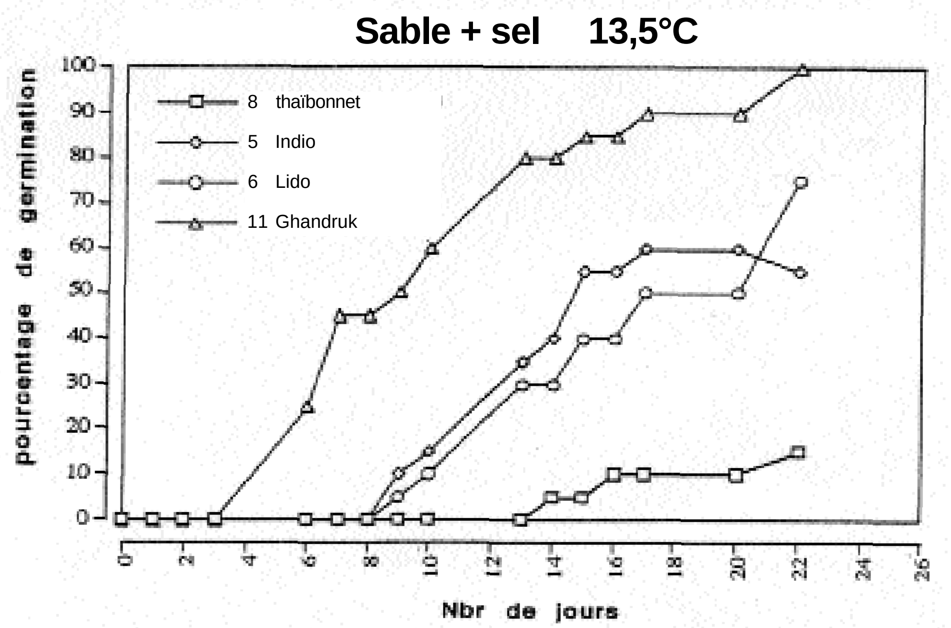
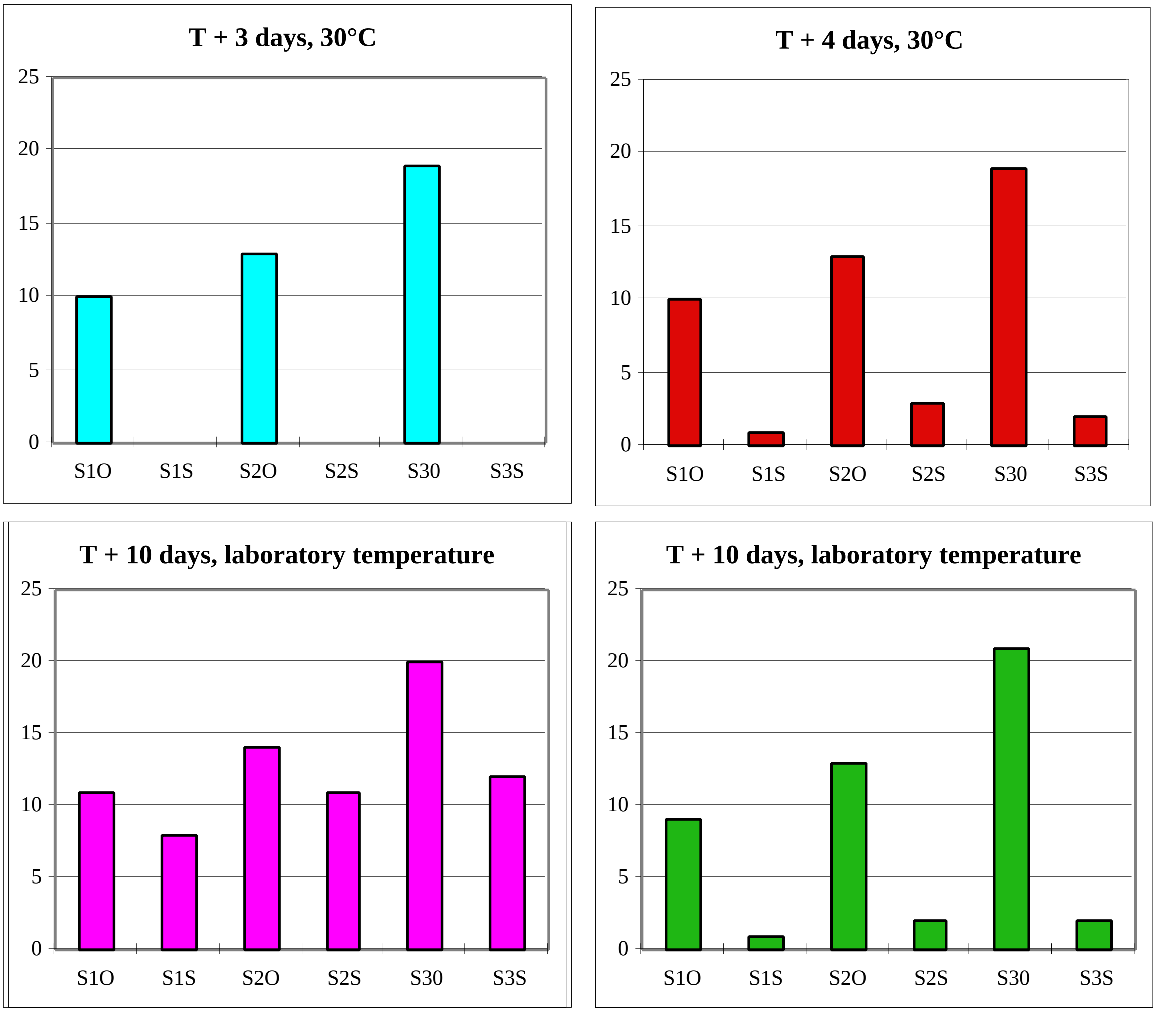


Figure 9. Number of germinated seeds in distilled water versus 2,7 g/l Na Cl solution (sample of 25 seeds Petri dish)

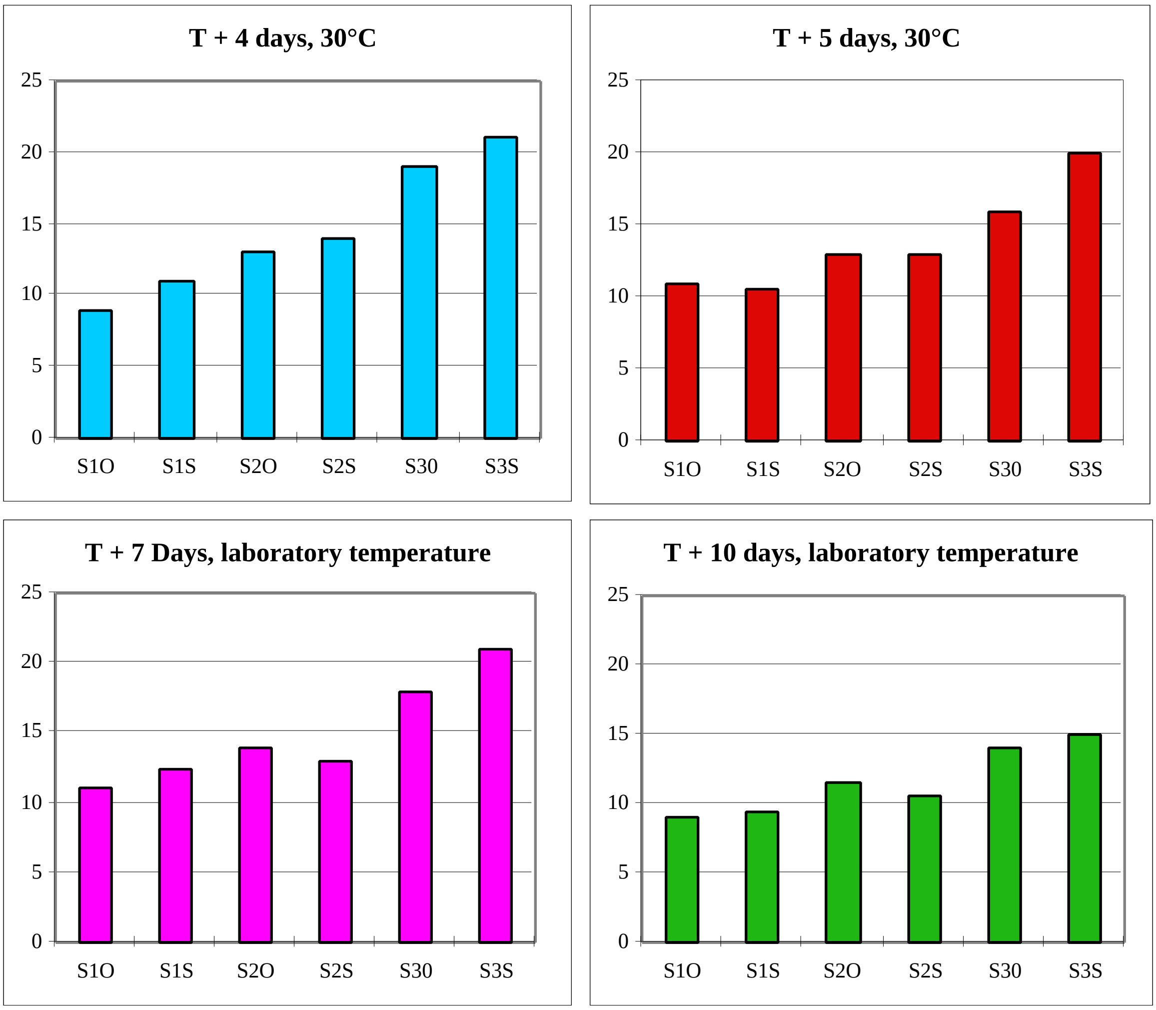


LEGEND

Seeds multiplication condition
S1 = Weakly salted
S2 = moderate salted
S3 = strongly salted

Seeds germination conditions
S*0 = distilled water
S*S = 10 g / l Na Cl solution

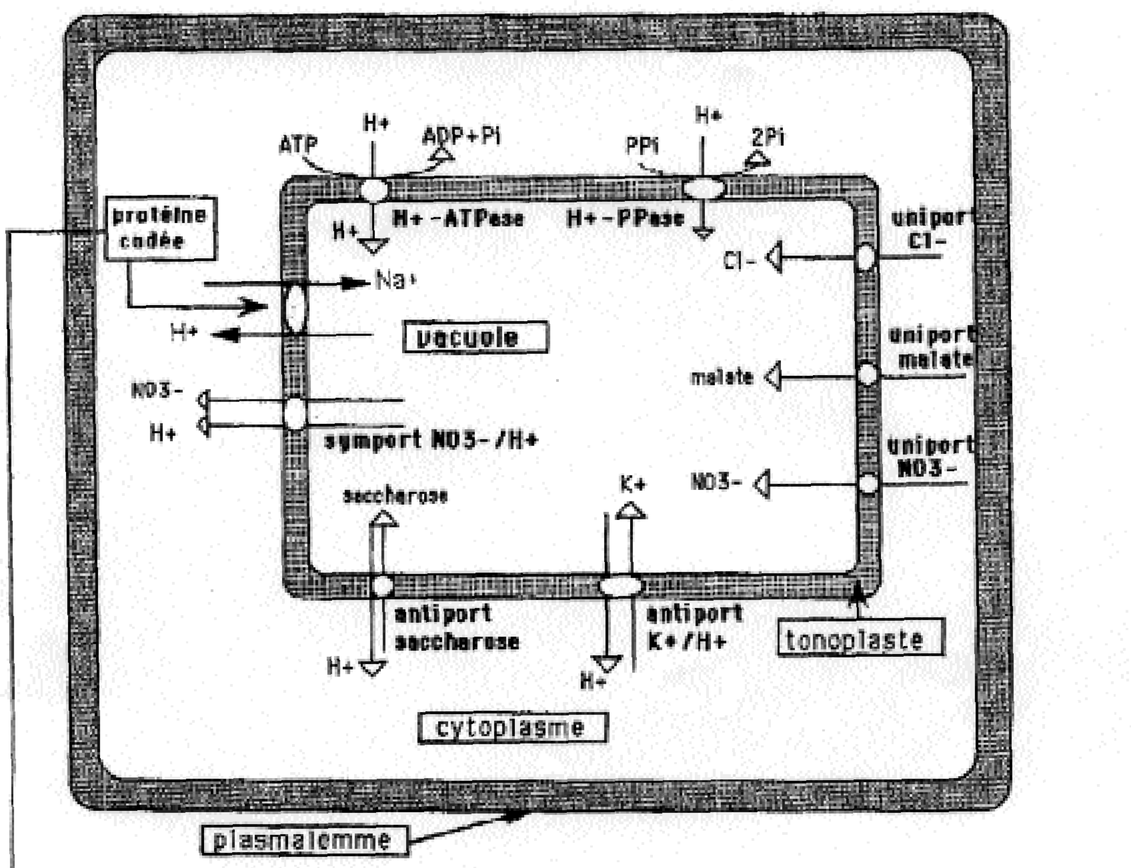
Figure 10. Number of germinated seeds in distilled water versus 10 g/l Na Cl solution (sample of 25 seeds Petri dish)



LEGEND

Seeds multiplication condition	**Seeds germination conditions**
S1 = Weakly salted	S*0 = distilled water
S2 = moderate salted	S*S = 2,7 g / l Na Cl solution
S3 = strongly salted	

Figure 11. Salt adaptation machanism in cells



A 69 K Dalton coded protein, located in the tonoplast, allows the Na+ absorption. In this situation, the absorption rates are of 8 for Na+ and 6 for Cl-. The obtained osmotic adjustment between the cytoplasm and the vacuole is the origin of salt tolerance.