# Physiological Studies on Seed Priming of Rice for Establishment in Stressed Environment

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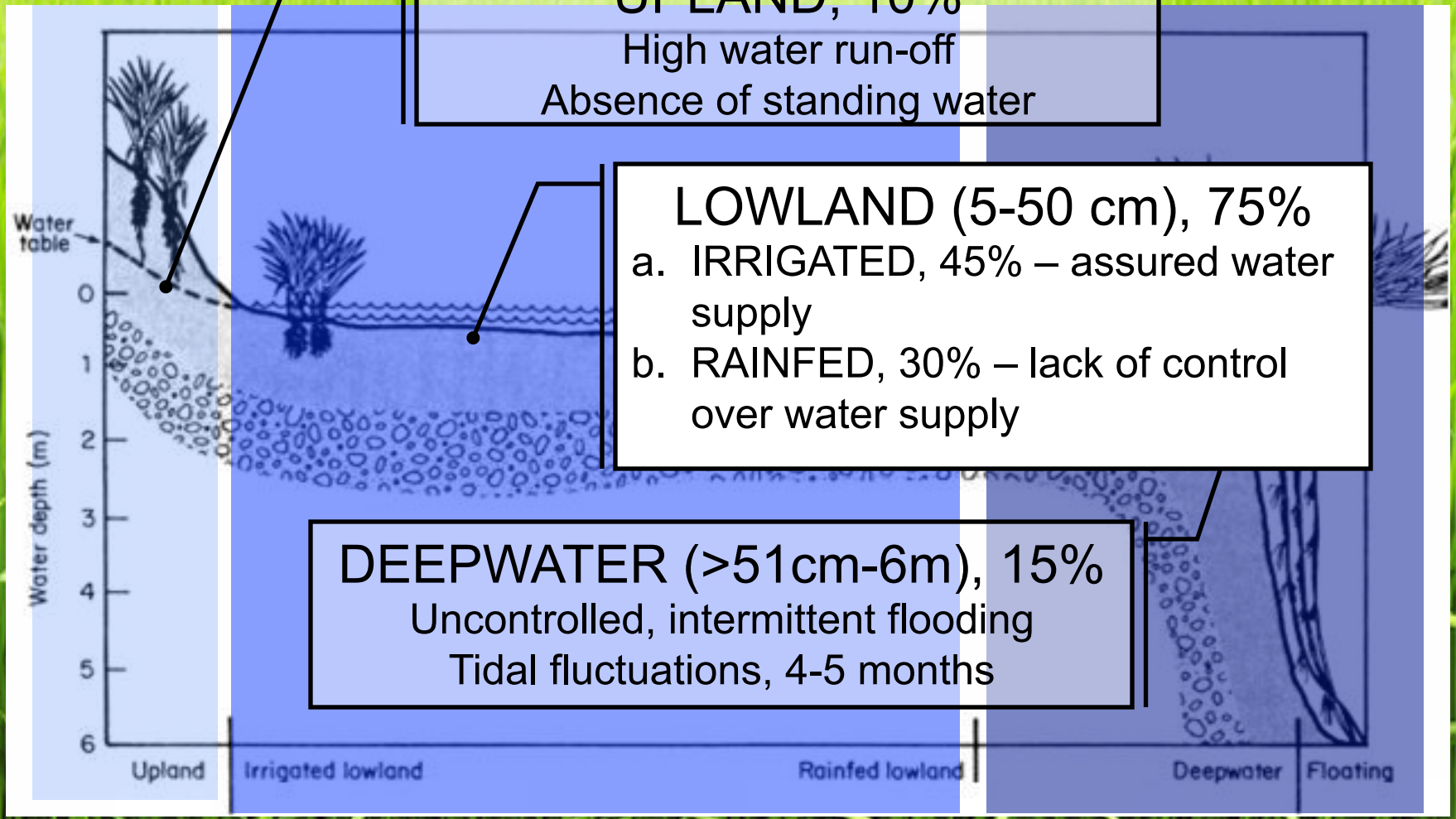
# Rice is an important cultivated cereal

http://foodportraits.com/food-culture/rice-asian-ingredient

Source: © 2006 Juan Lazaro IV, International Rice Research Institute

http://www.irri.org/index.php?option=com_k2&view=item&id=9081&Itemid=100481

# RICE ECOTYPES
# Based on Soil Surface Hydrology




**UPLAND, 10%**
High water run-off
Absence of standing water

**LOWLAND (5-50 cm), 75%**
a. IRRIGATED, 45% – assured water supply
b. RAINFED, 30% – lack of control over water supply

**DEEPWATER (>51cm-6m), 15%**
Uncontrolled, intermittent flooding
Tidal fluctuations, 4-5 months

De Datta, 1981

# FLOODING events have increased across the globe

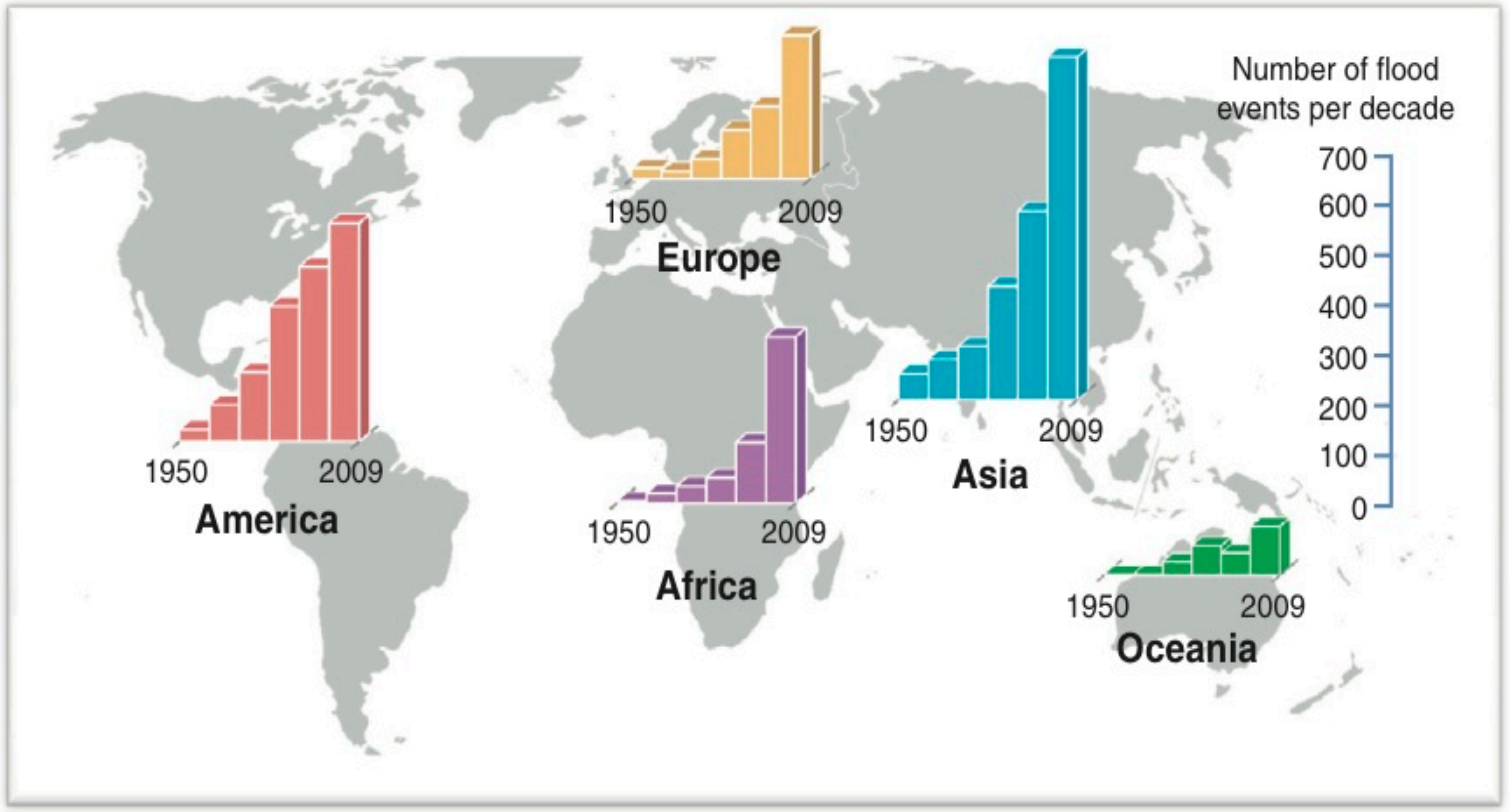
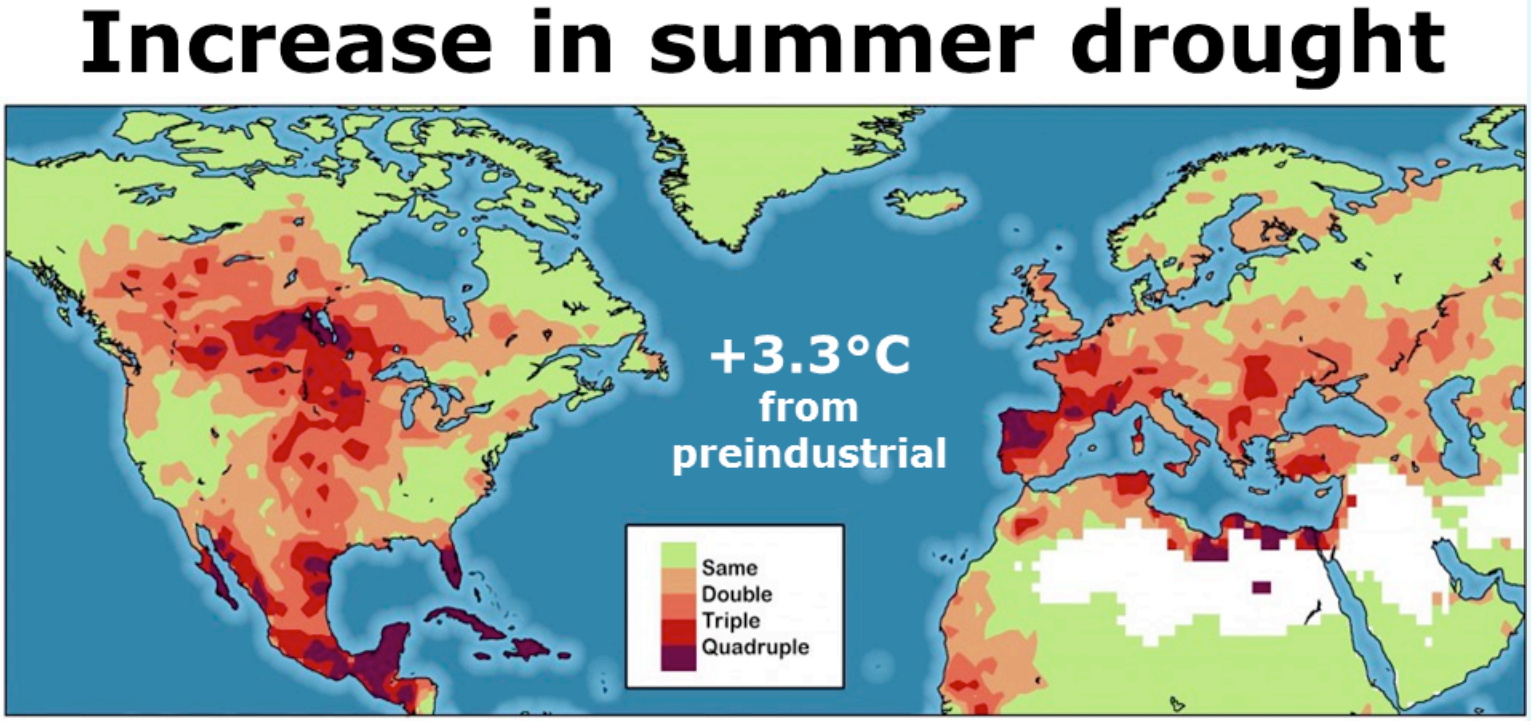



**Figure 1**. Numbers of floods have increased in each of the past six decades across the globe. Graphs show the number of floods classified as a disaster in the International Disaster Database of the University of Louvain, Belgium for the period from 1950 through 2009 by geographical region [93]. Events include river or coastal floods, rapid snow melts, heavy rainfall and other occurrences that caused significant social or economic hardship. Adapted from a Millennium Ecosystem Assessment map (http://maps.grida.no/go/graphic/number-of-flood-events-by-continent-and-decade-since-1950).

# WATER DEFICIT/DROUGHT

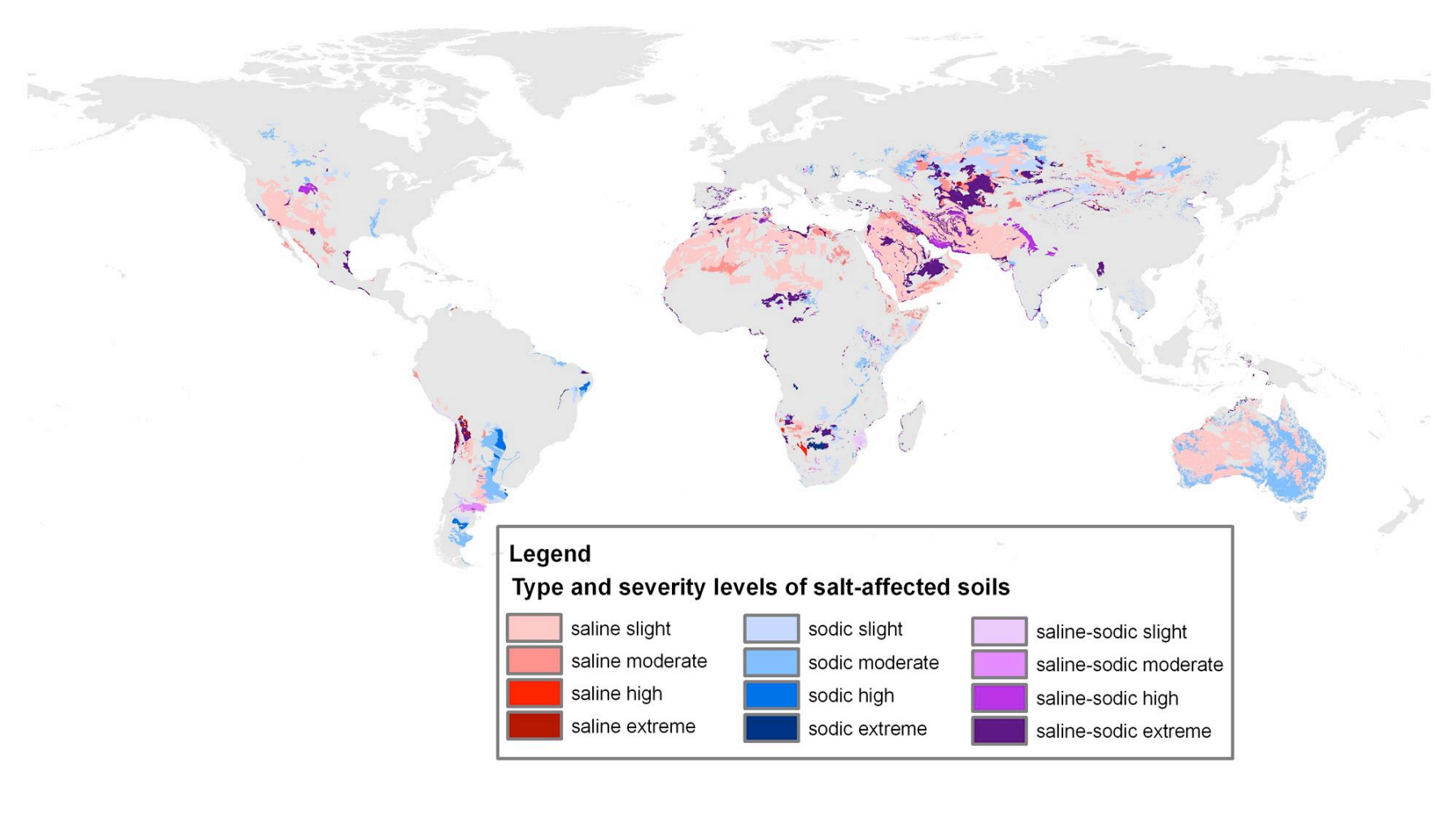



*Change in the likelihood of summer season drought (defined as a once in ten years event for the late 20th century) by the end of the 21st century, according to multi-decade simulations with the operational model of the European Centre for Medium-range Weather Forecasts (ECMWF) based on the A1b scenario of the IPCC.*

Floods and Droughts in a Changing Climate – Now and the Future
*April 29th, 2011* Paul A. Dirmeyer
*Center for Ocean-Land-Atmosphere Studies*
*Calverton, Maryland*

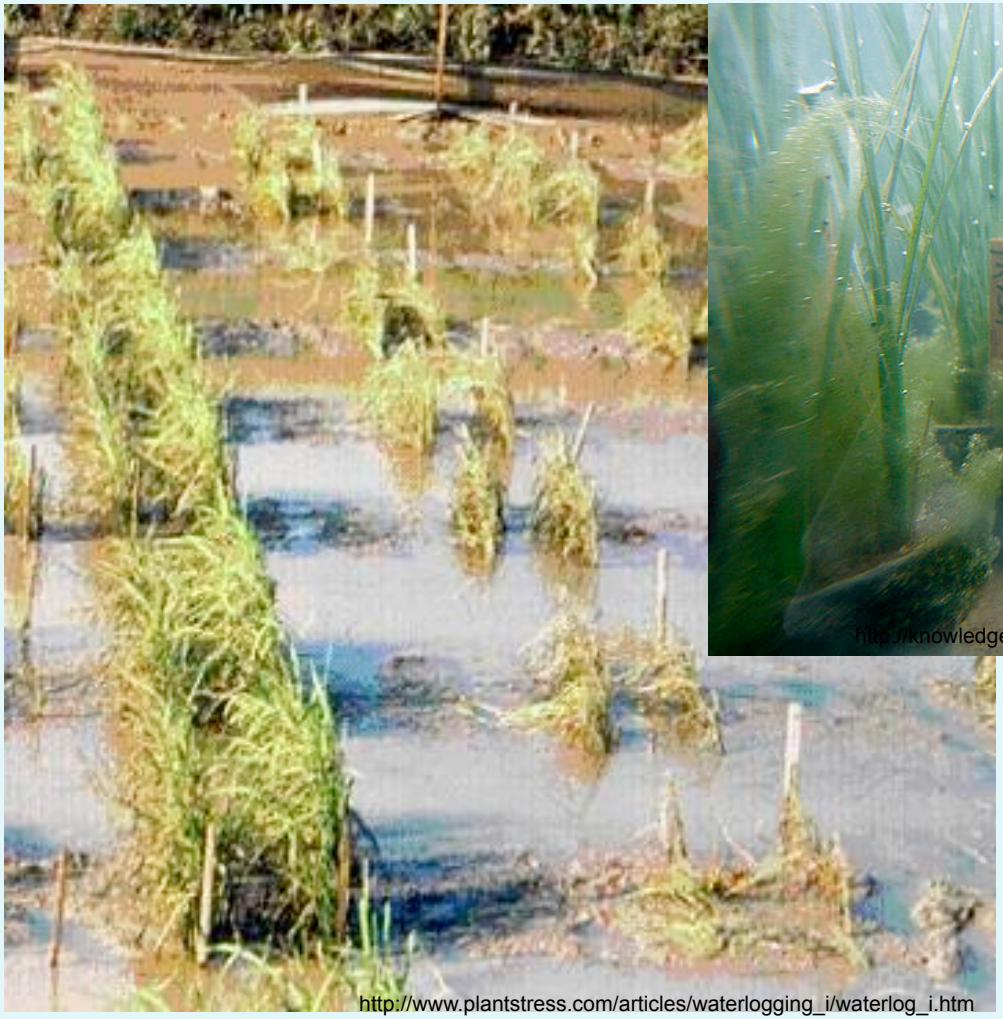
http://www.climatechange-foodsecurity.org/uploads/drought_likelihood.png

# SALINITY STRESS




http://www.clubgreen.nl/vraag/Biosaline-agroforestry-and-forestry-world-large.jpeg

# FLOODING

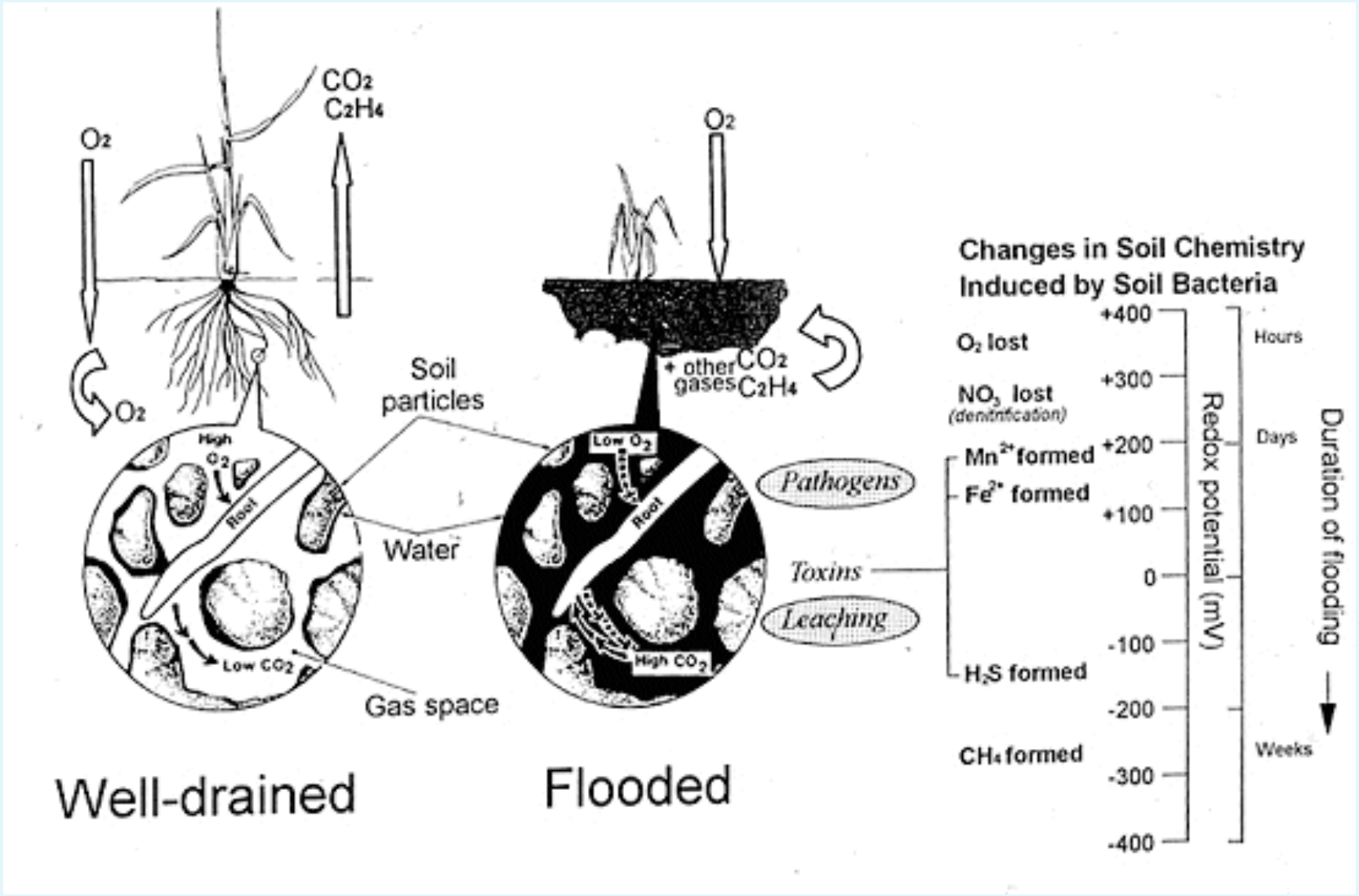


http://www.plantstress.com/articles/waterlogging_i/waterlog_i.htm

WATERLOGGING


SUBMERGENCE

# Associated Problems in Flooded Soil

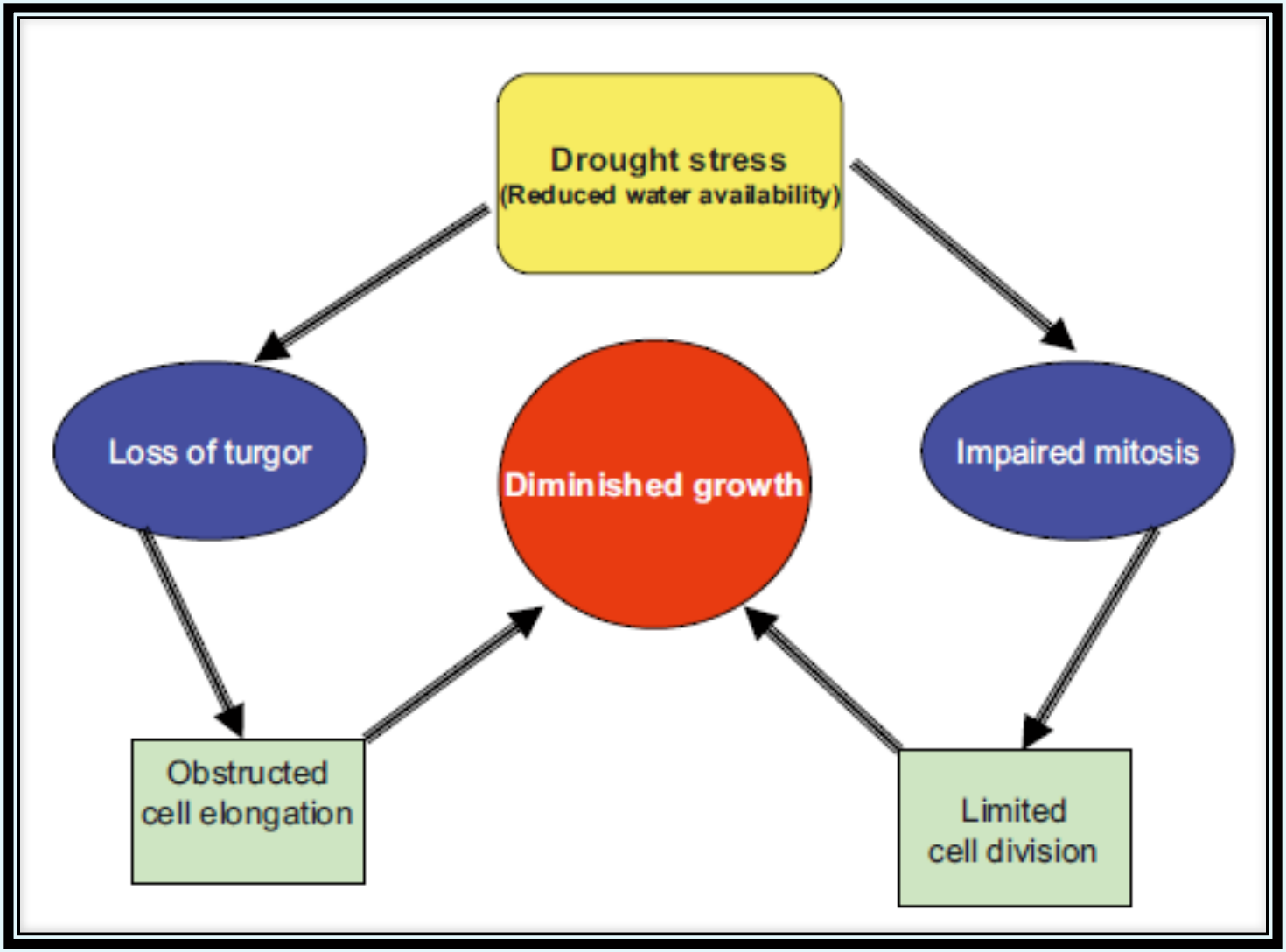



http://www.plantstress.com/articles/waterlogging_i/waterlog_i.htm

# WATER DEFICIT/DROUGHT

http://www.knowledgebank.irri.org/ricebreedingcourse/image113.jpg

# Associated Problems Related to Drought




Farooq et al. 2009

# SALINITY STRESS

- **Single, most widespread soil toxicity problem**
- **Substantial agricultural lands lost annually to salinity**
- **Problem on the rise due to climatic changes and bad agricultural practices**

# Associated Problems Related to Salinity

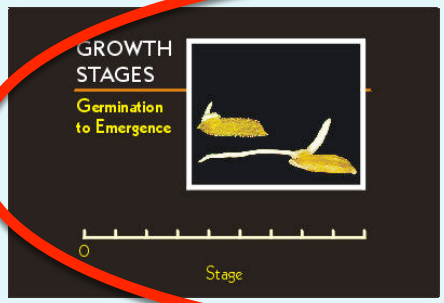
- **Osmotic Stress**

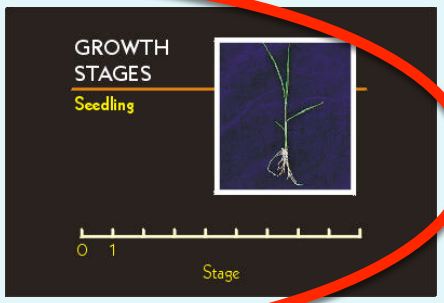
**High salt concentration**
↓
**Low water potential**
↓
**Reduced water absorption**
↓
**Water deficit**

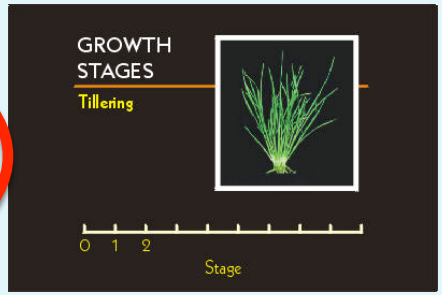
- **Ionic Stress**

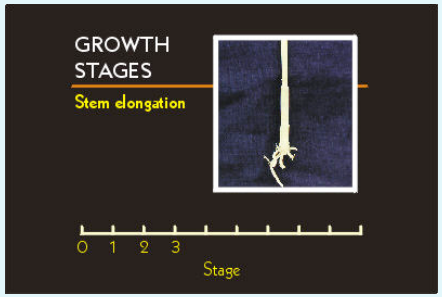
**High salt concentration**
↓
**Ion ($Na^+$ / $Cl^-$) toxicity**
↓
**Reduced $K^+$ uptake**
↓
**Nutrient imbalance**

# Rice Growth Stages

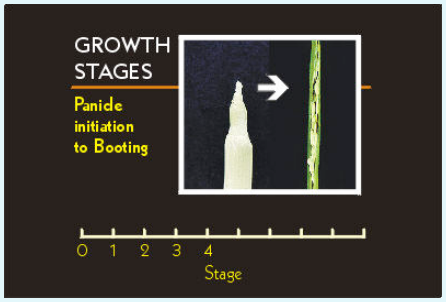


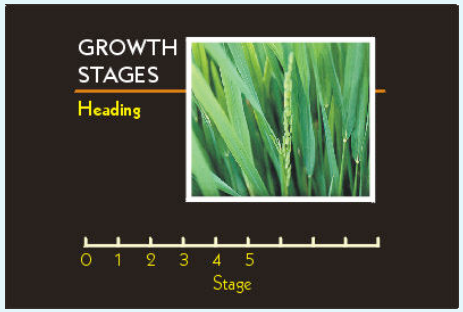



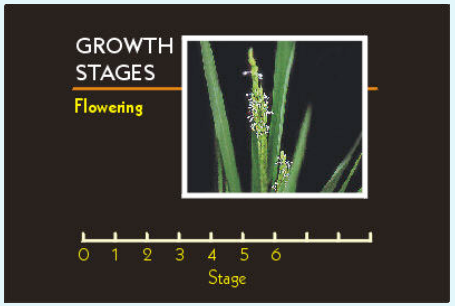



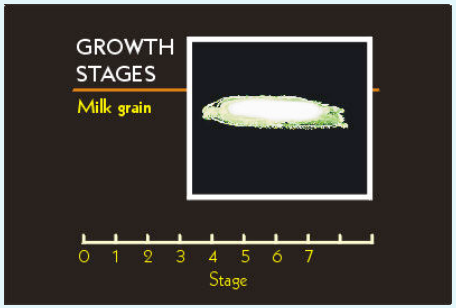

## Vegetative phase

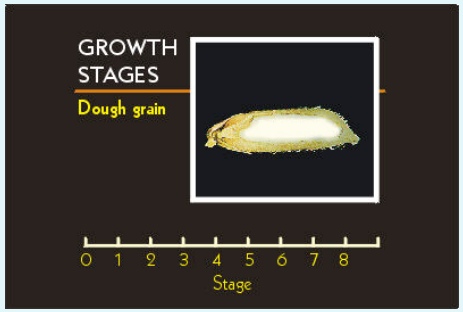


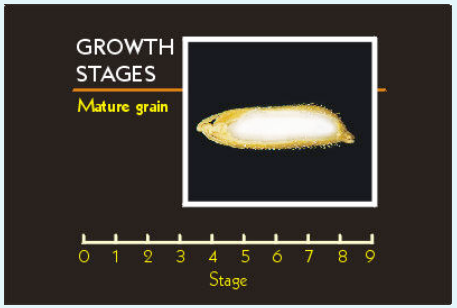




## Reproductive phase



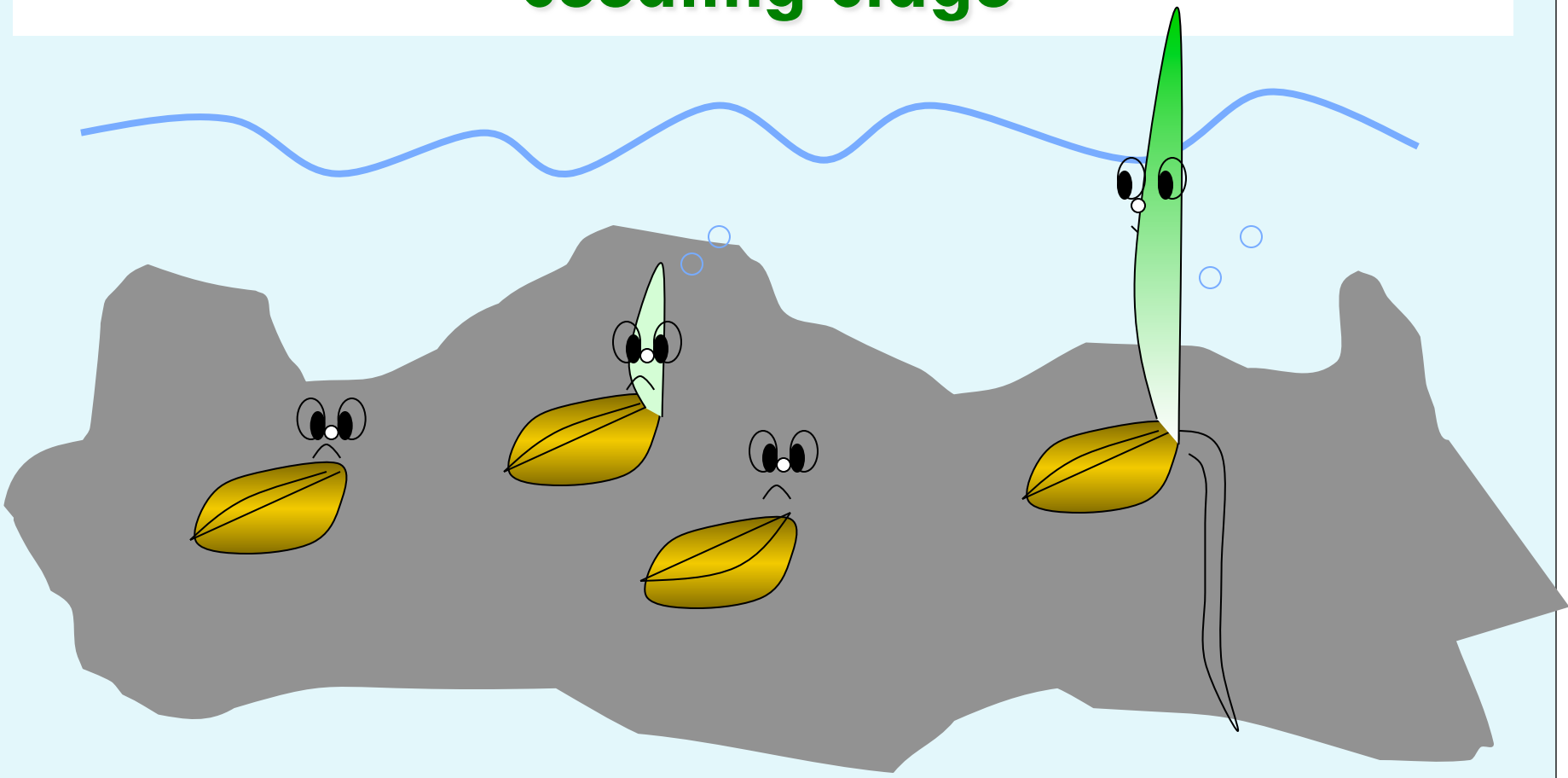



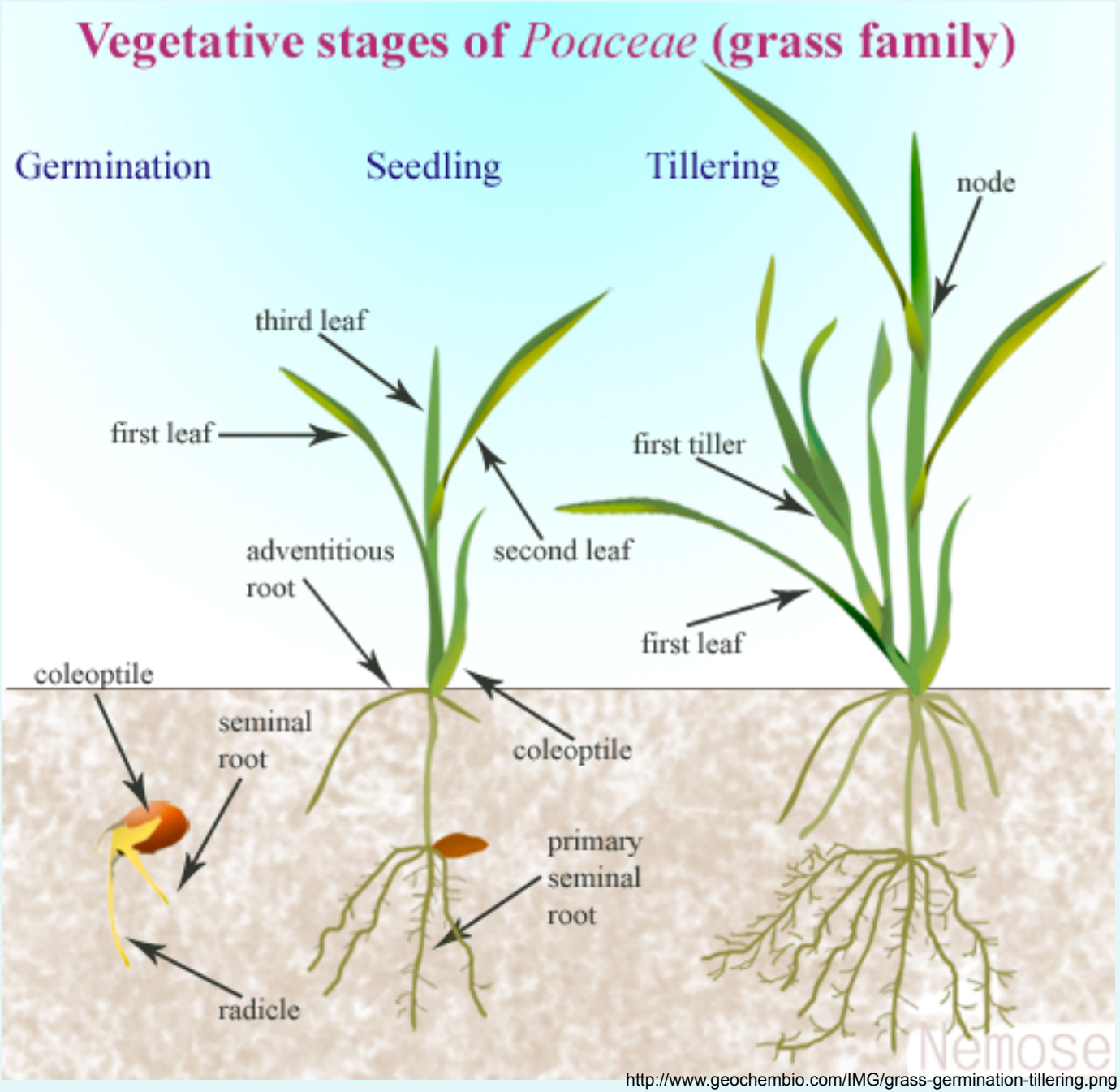




## Ripening phase

http://www.knowledgebank.irri.org/extension/growth-stages-0-9.html

# Flooding, drought and salinity mutually affect seed germination and early seedling stage





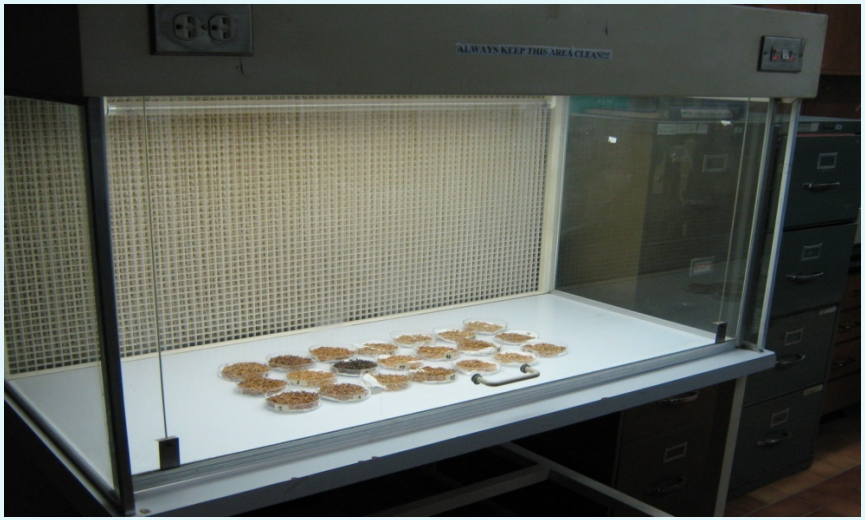

http://www.geochembio.com/IMG/grass-germination-tillering.png

# Seed Priming

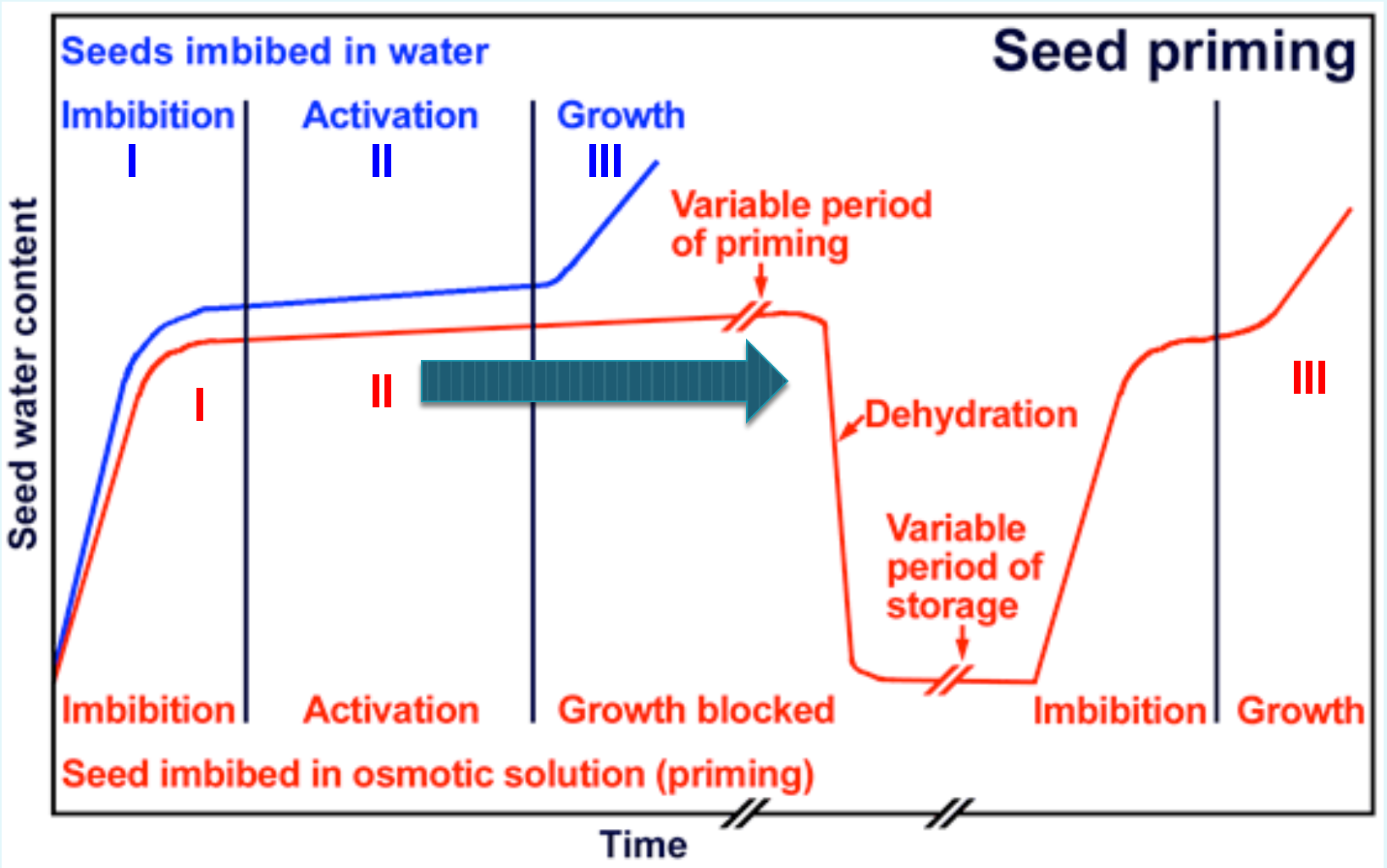
- Simple, low-cost, low-risk seed invigoration strategy
- Improve tolerance to undesirable germination and early seedling establishment
- Known to enhanced performance of several horticultural crops
- Effect on rice crop subjected to stressful environment not fully explored

# Seed priming

- Uptake of water to initiate early stages of germination
- No radicle protrusion
- Drying to the original seed moisture content

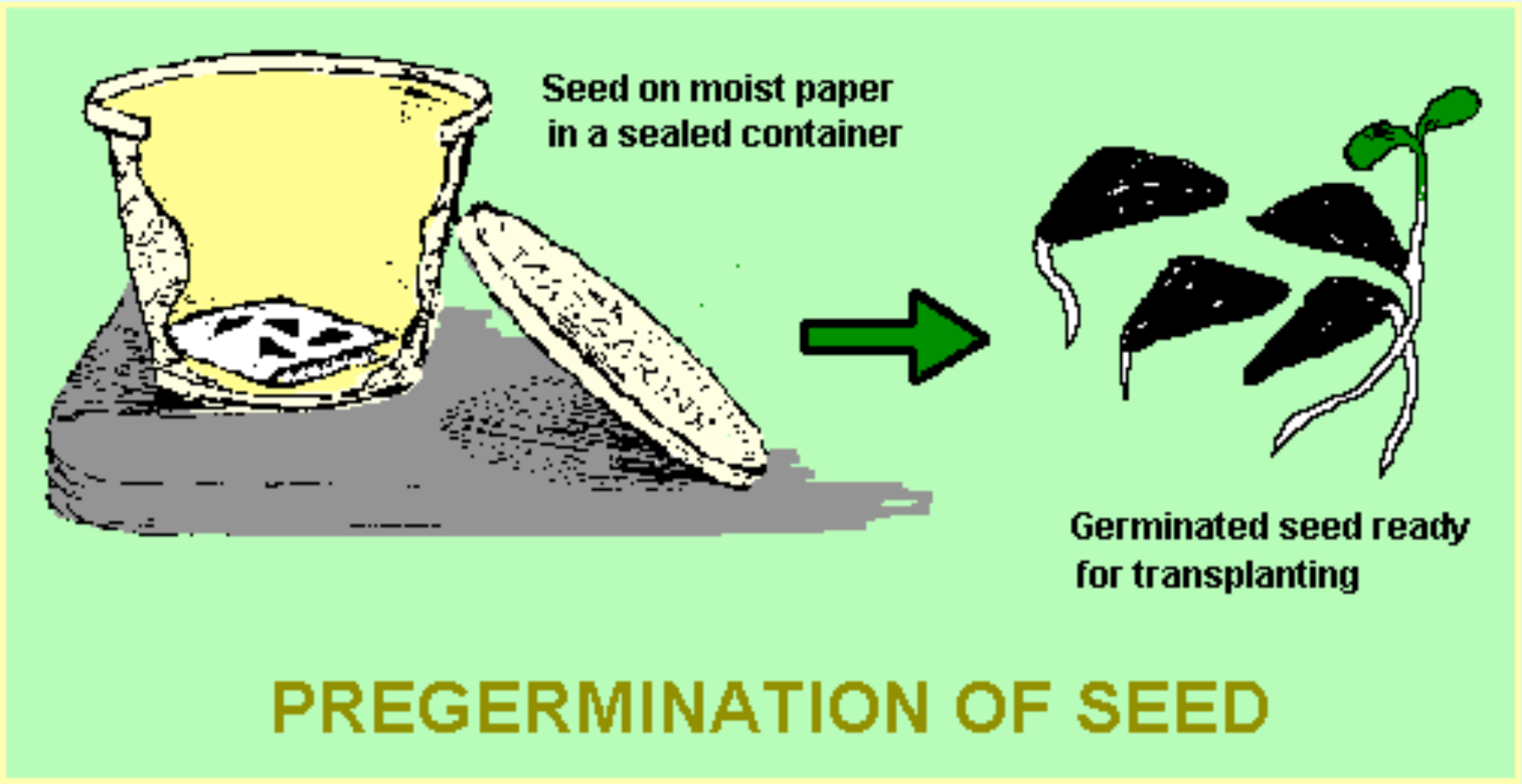
# Physiology of Seed Priming





Bradford KJ, Bewley JD (2002). Seeds: Biology, Technology and Role in Agriculture. Chapter 9, pp. 210-239. In: Plants, Genes and Crop Biotechnology (eds Chrispeels MJ, Sadava DE), Jones and Bartlett, Boston.

http://www.seedbiology.de/images/priming-1.gif




http://anpsa.org.au/gif/pregerm1.gif

# Methods (Hydration) of Seed Priming

- **Hydropriming** water
- **Osmopriming** osmotic solution (inorganic salts or PEG)
- **Matripriming** non-toxic solid carrier (vermiculite, peat moss)

# Effect of seed priming on rice seeds and seedlings subjected to stress

- Priming methods
- Germination and seedling establishment
- Cultivars contrasting in tolerance
- Carbohydrate mobilization and membrane lipid peroxidation

# MODES OF RICE CROP ESTABLISHMENT

TRANSPLANTING

DRY

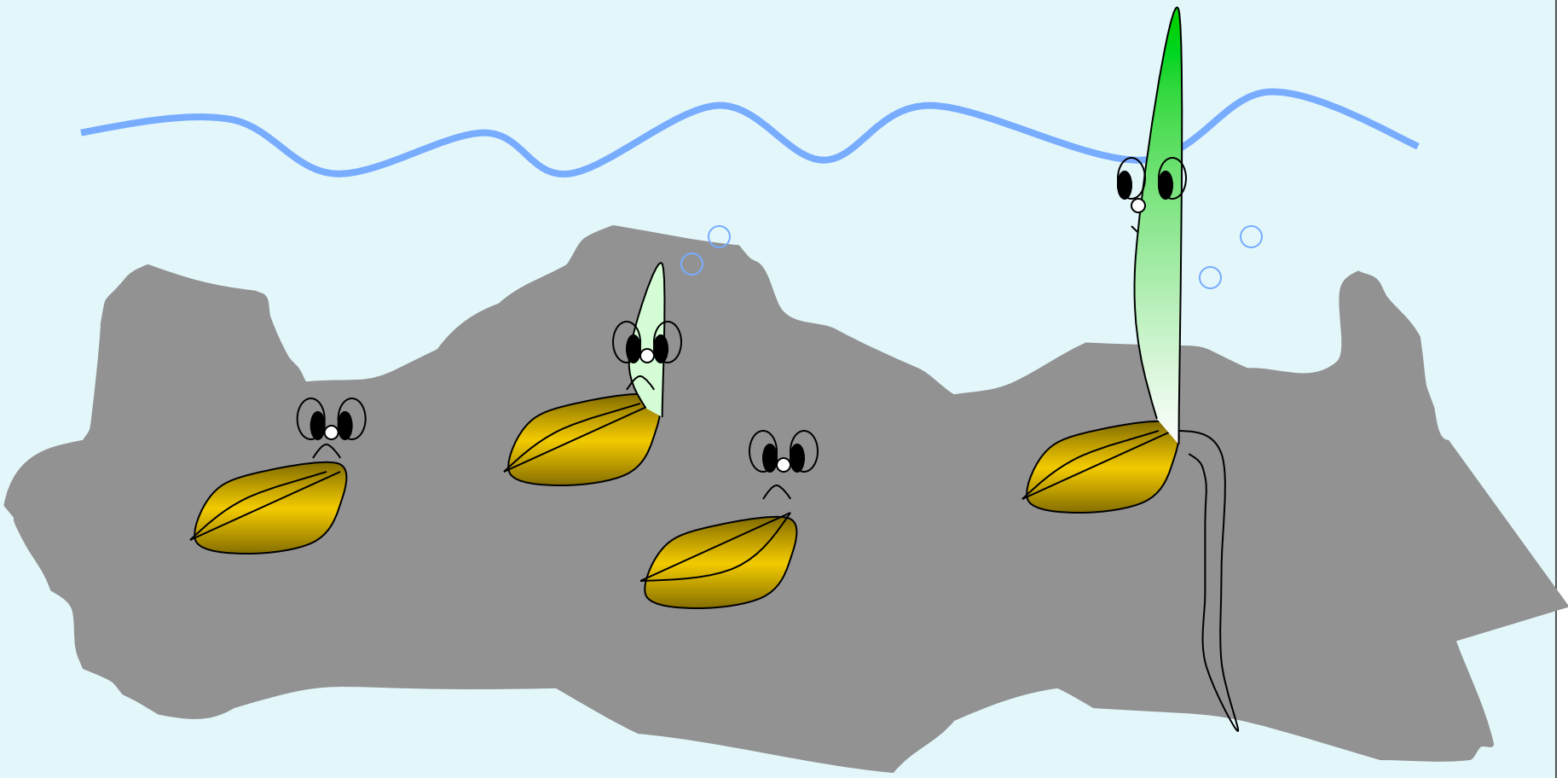
WET

DIRECT SEEDING

# Advantages of Direct Seeding

- Reduction on labor cost
- Results in earlier harvest
- Promotes crop intensification
- Reduction in irrigation requirements
- Reduction on herbicide use (early flooding as weed control)

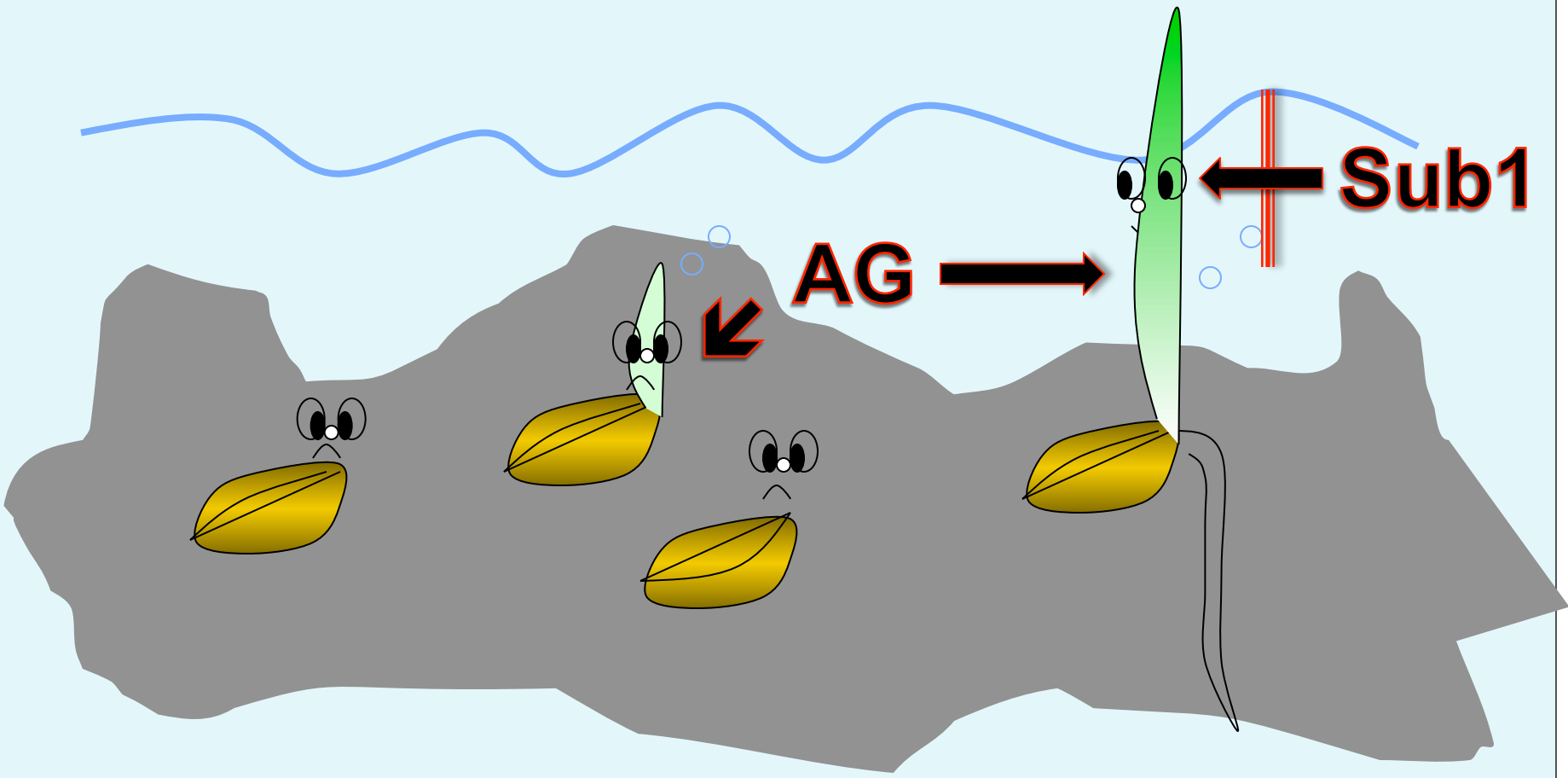
# Early flooding requires tolerance during germination in direct–seeded rice



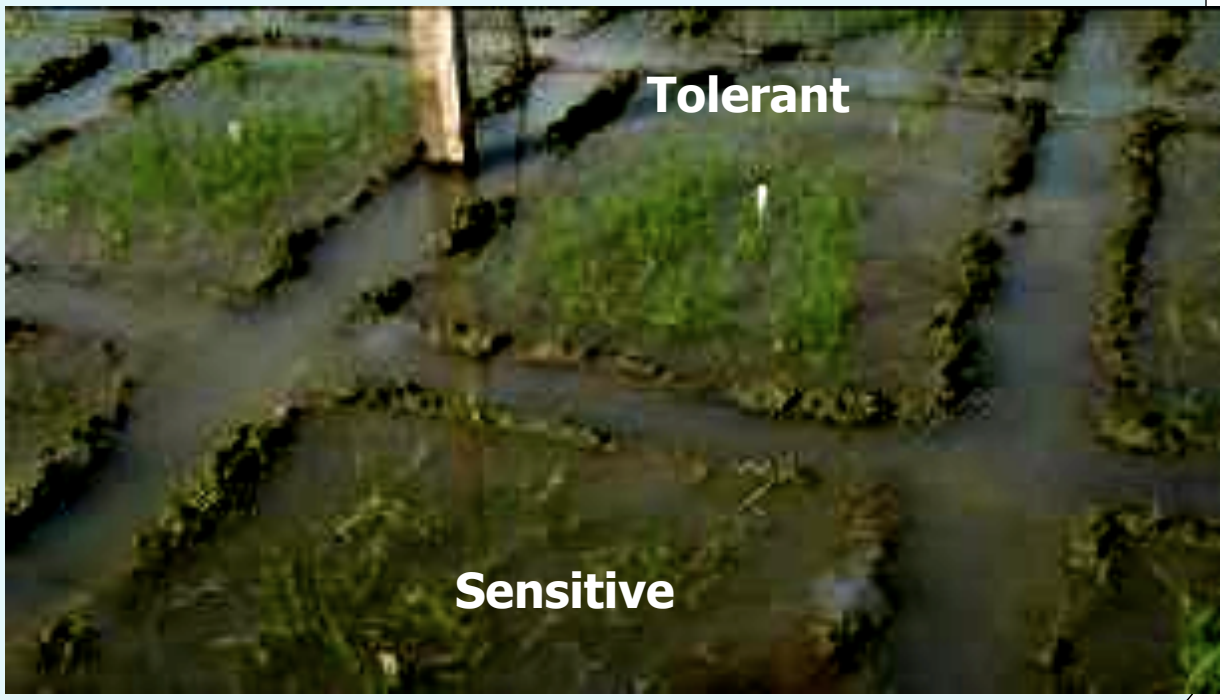
# Rice Genotypes

| Grouping | Genotype | Relevant Tolerance |
|---|---|---|
| AG-Parental Lines | Khao Hlan On (KHO)<br>Ma Zhan Red (MZR)<br>Khaiyan | Tolerant |
| AG-Derived Lines | IR87181-87-2-1<br>IR81935-33-1-2-1<br>IR817181-87-2-1<br>IR83770-9-3-2-23 | |
| Non-AG, Non-sub1 Lines | IR42, IR64<br>BR11, CR1009, PSBRc10<br>Swarna, Sambha Mahsuri | Intolerant |
| Non-AG, sub1 Lines | IR64 sub1, BR11 sub1<br>CR1009 sub1, Swarna sub<br>Sambha Mahsuri sub1<br>IR66876-11-NDR-1 sub1<br>INPARA-3, PSBRc68 | |

# Early flooding requires tolerance during germination in direct–seeded rice




Khaiyan
Khao Hlan On
IR42


Tolerant
Sensitive

# Priming Procedure and Sowing Condition


**Soaking in Water or Salt Solution for 12h / 24h / 48h**

**Forced Air-Drying for 12h / 24h**

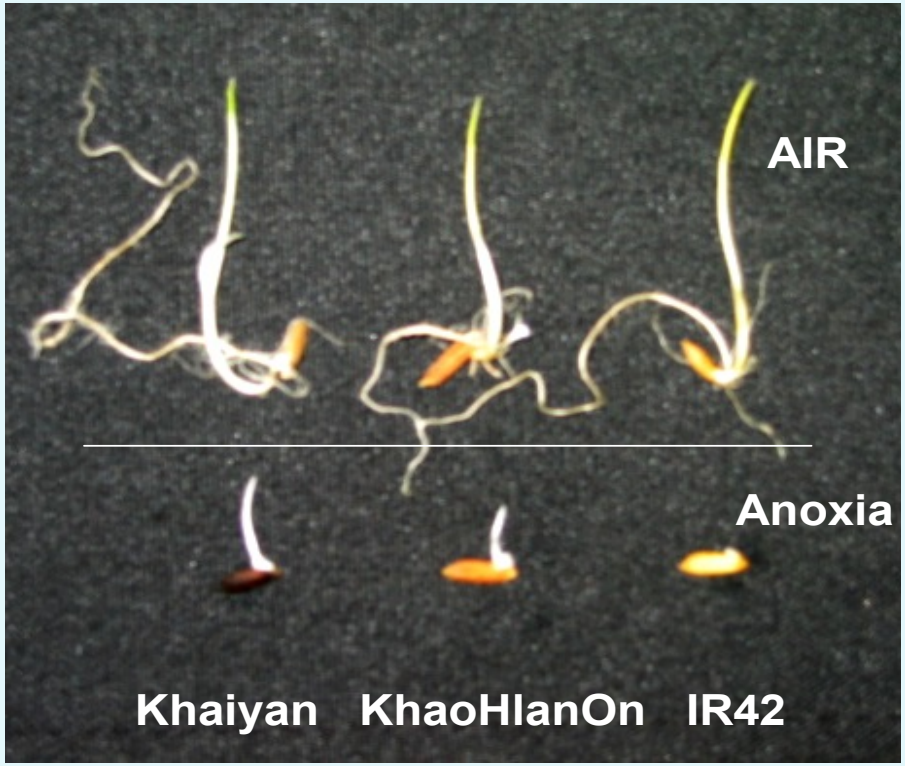
**Laboratory Condition Deoxygenated Stagnant Agar**

**Greenhouse Condition Flooded Soil**

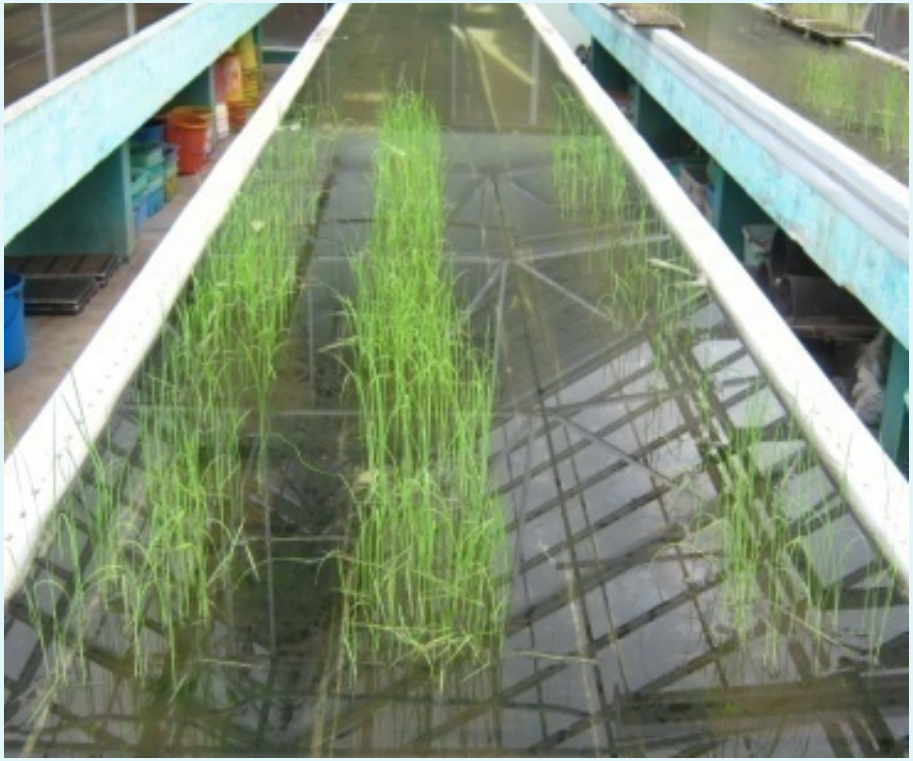
# Parameters Measured

- Deoxygenated Agar Condition
  - Germination percentage
  - Coleoptile length
- Flooded Soil Condition
  - Germination percentage (survival)
  - Shoot length
  - Mesocotyl length
  - Root length

# Germination and Survival




**Seven Days (7d) after Sowing in Deoxygenated Agar Condition**



**Twenty-one (21d) after Sowing in Flooded Soil Condition**

# Priming Effect on Seed Germination in Hypoxic Condition

| Genotype | Unprimed | Hydropriming | | Osmopriming | |
|---|---|---|---|---|---|
| | | 12h:12h | 24h:24h | 12h:12h | 24h:24h |
| AG-Parental Lines (Tolerant) | Germination Percentage | | | | |
| KHO | 100.0 | 98.3 | 100.0 | 98.3 | 93.3 |
| MZR | 100.0 | 95.0 | 100.0 | 95.0 | 90.0 |
| Khaiyan | 95.0 | - | 95.0 | - | 98.3 |
| AG-Derived Lines | | | | | |
| IR81159 | 85.0 | 95.0 | - | 95.0 | - |
| IR81935 | 98.3 | 98.3 | 98.3 | 95.0 | 93.3 |
| IR87181 | 80.0 | 90.0 | 90.0 | 90.0 | 93.3 |
| IR83770 | 96.67 | - | 98.3 | - | 91.67 |
| Non-AG Lines (Sensitive) | | | | | |
| IR42 | 58.3 | 96.67 | 98.3 | 96.67 | 95.0 |
| IR64 | 65.0 | - | 100.0 | - | 98.3 |

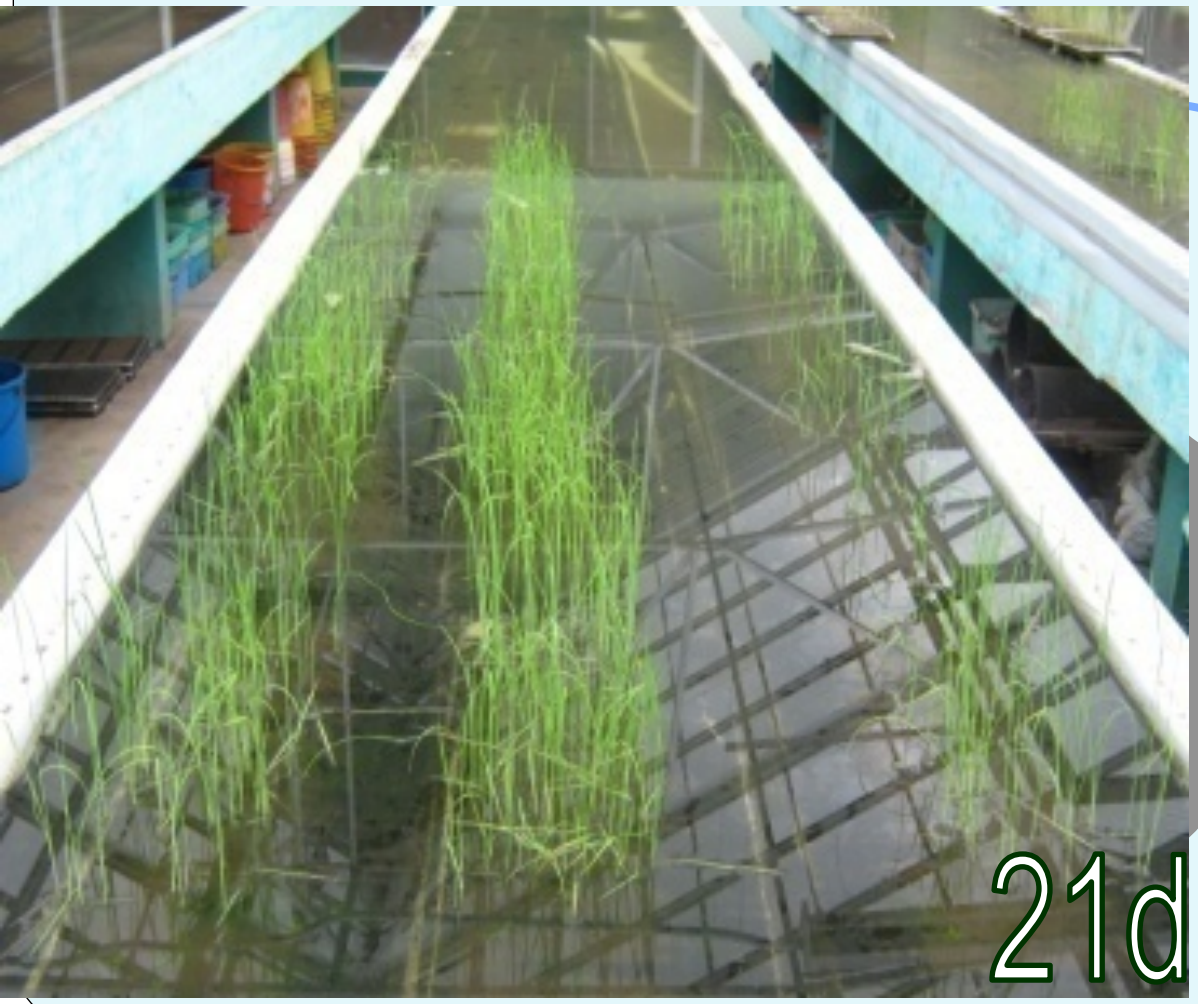
- **Seed priming significantly improved germination of sensitive genotypes**

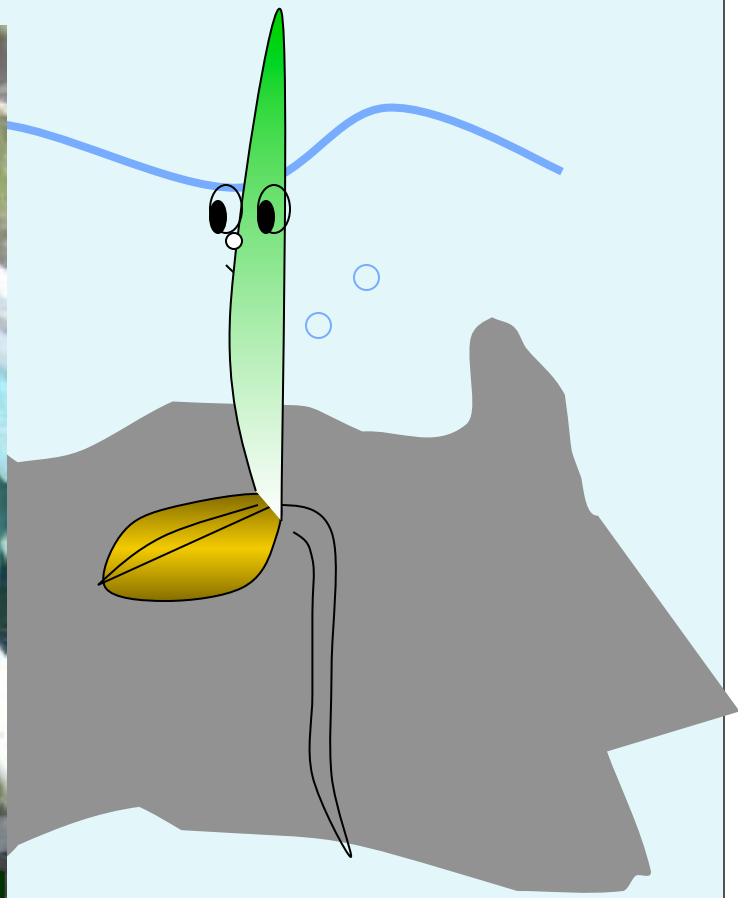
# Priming Effect on Coleoptile Length in Hypoxic Condition

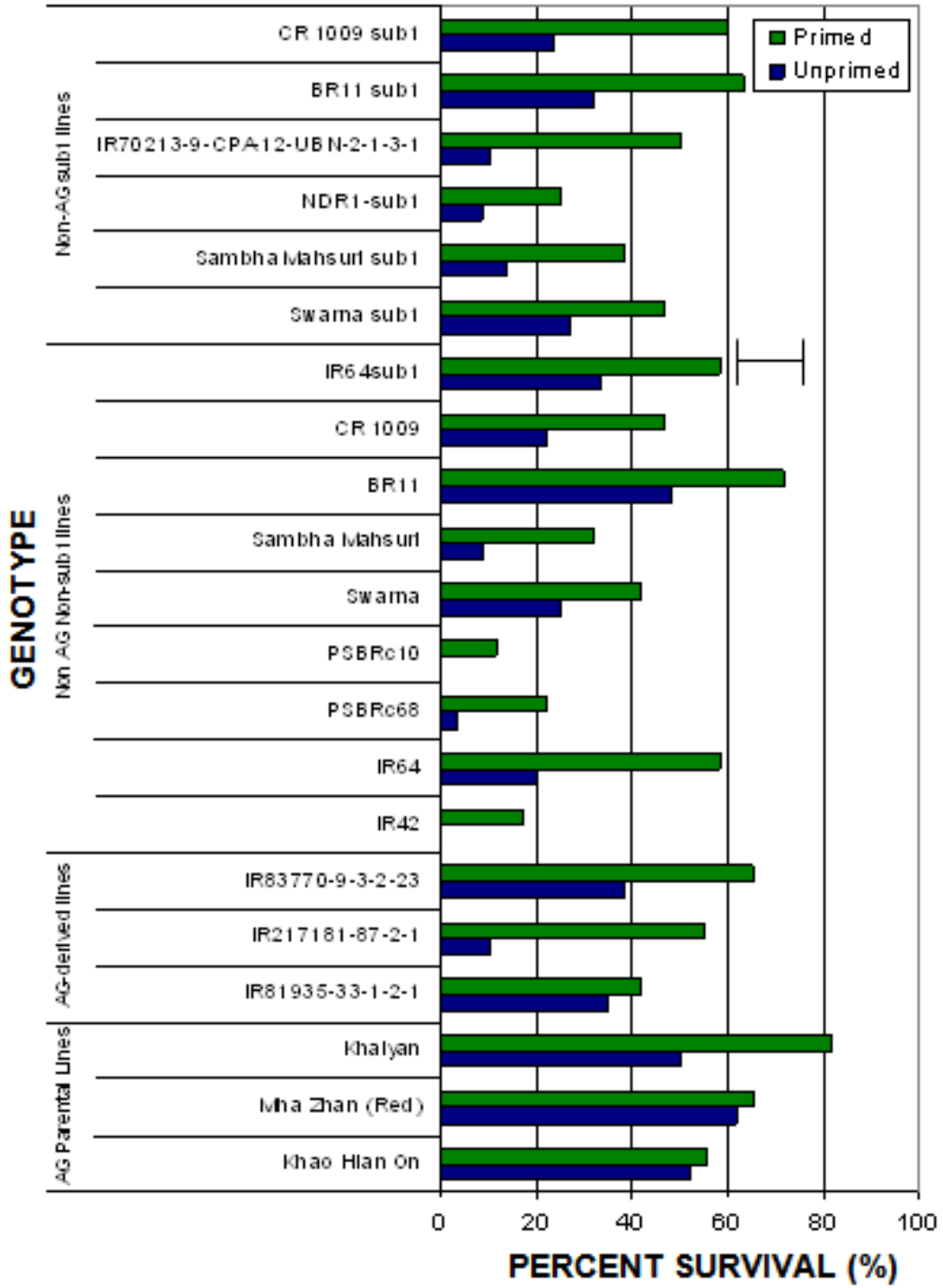
| Genotype | Unprimed | Hydropriming | | Osmopriming | |
|---|---|---|---|---|---|
| | | 12h:12h | 24h:24h | 12h:12h | 24h:24h |
| AG-Parental Lines (Tolerant) | Coleoptile Length (cm) | | | | |
| KHO | 1.71 | 2.31 | 1.42 | 1.34 | 1.07 |
| MZR | 1.92 | 1.34 | 1.51 | 2.36 | 1.12 |
| Khaiyan | 1.66 | - | 1.76 | - | 1.64 |
| AG-Derived Lines | | | | | |
| IR81159 | 0.70 | 0.92 | - | 0.88 | - |
| IR81935 | 1.18 | 1.75 | 1.27 | 1.59 | 0.87 |
| IR87181 | 0.89 | 0.97 | 0.90 | 1.67 | 1.01 |
| IR83770 | 1.07 | - | 1.19 | - | 0.79 |
| Non-AG Lines (Sensitive) | | | | | |
| IR42 | 0.09 | 0.45 | 0.89 | 0.22 | 0.80 |
| IR64 | 0.30 | - | 1.51 | - | 1.20 |

- Seed priming significantly improved coleoptile length especially of sensitive lines at longer soaking and drying duration

# In flooded soil, percentage germination was expressed in terms of survival

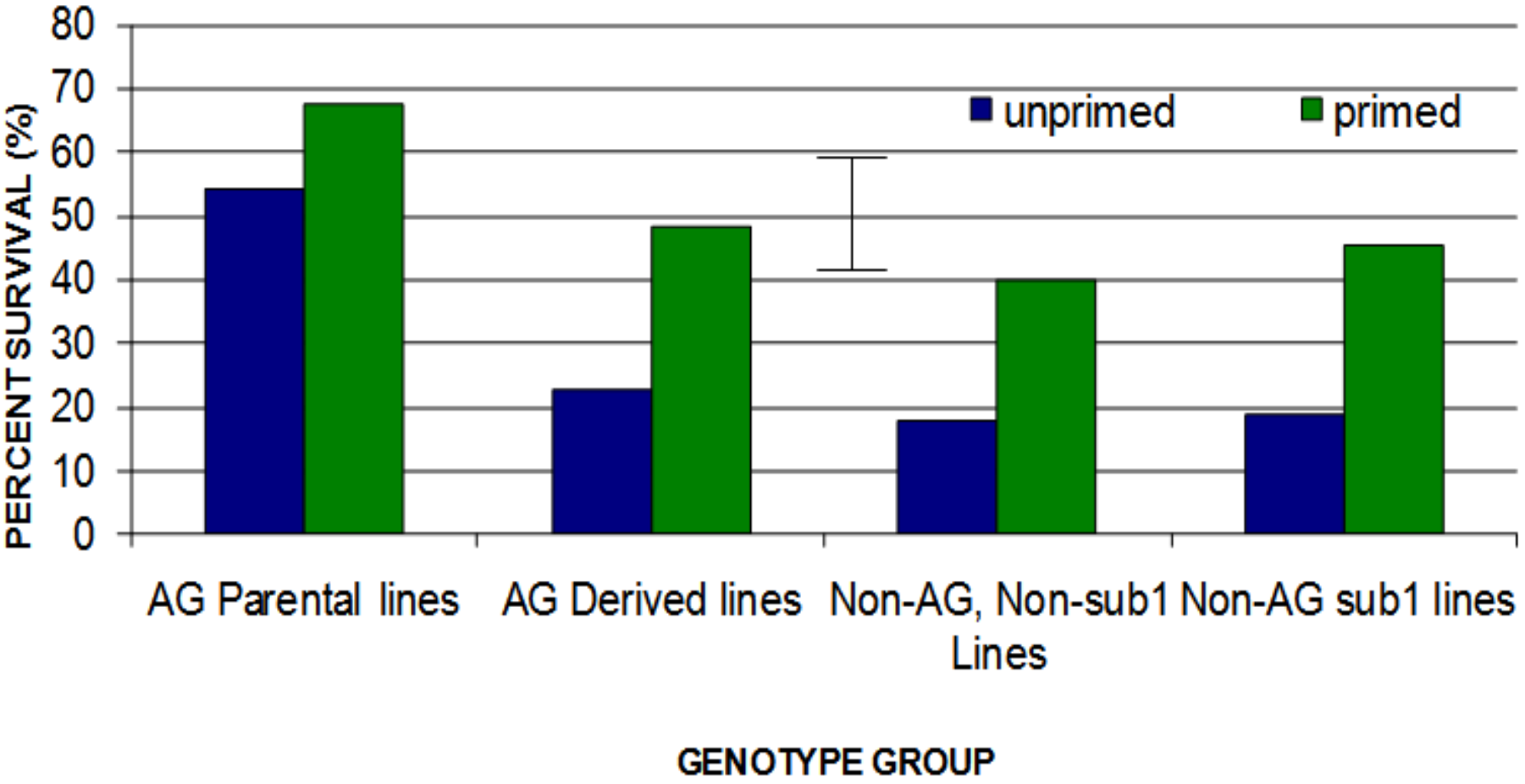




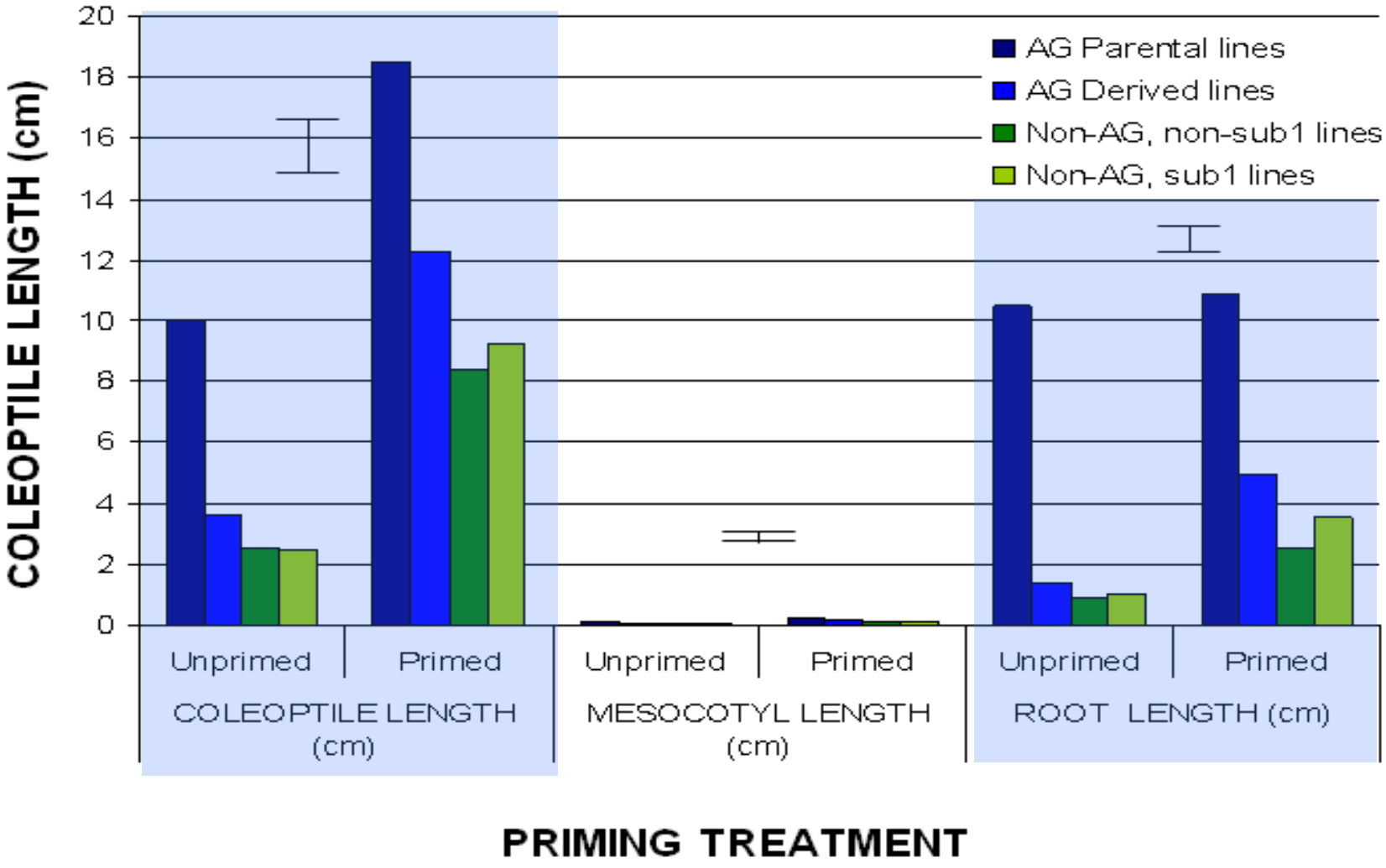

- **Seed priming significantly increased survival**
  - **All genotypes**
  - **More prominent in non-AG lines**

# Hydropriming (24h:24h) Effect on Survival in Flooded Soil

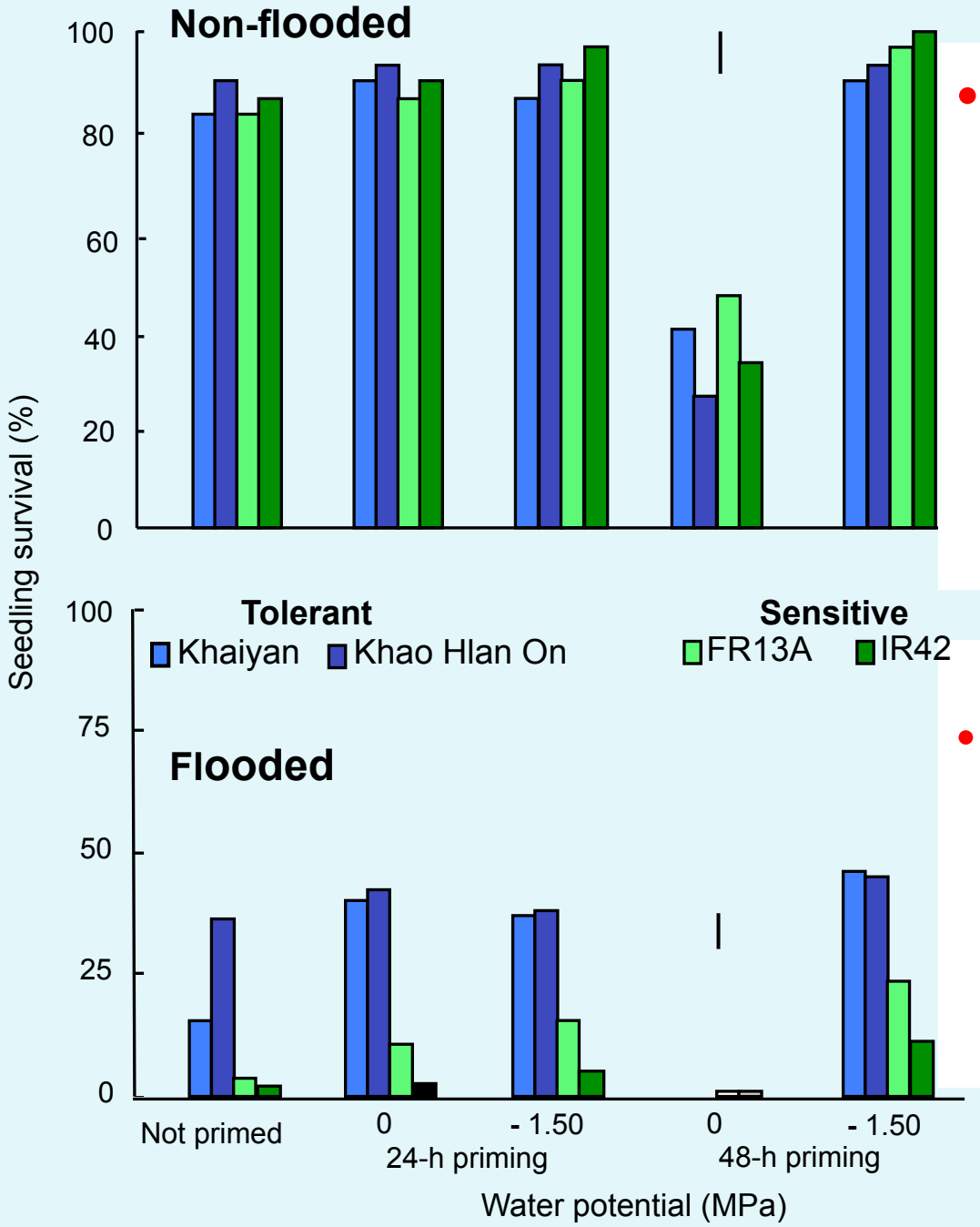



- **Hydropriming increased percentage survival of all genotypes**

# Hydropriming Effect on Shoot and Root Lengths in Flooded Soil



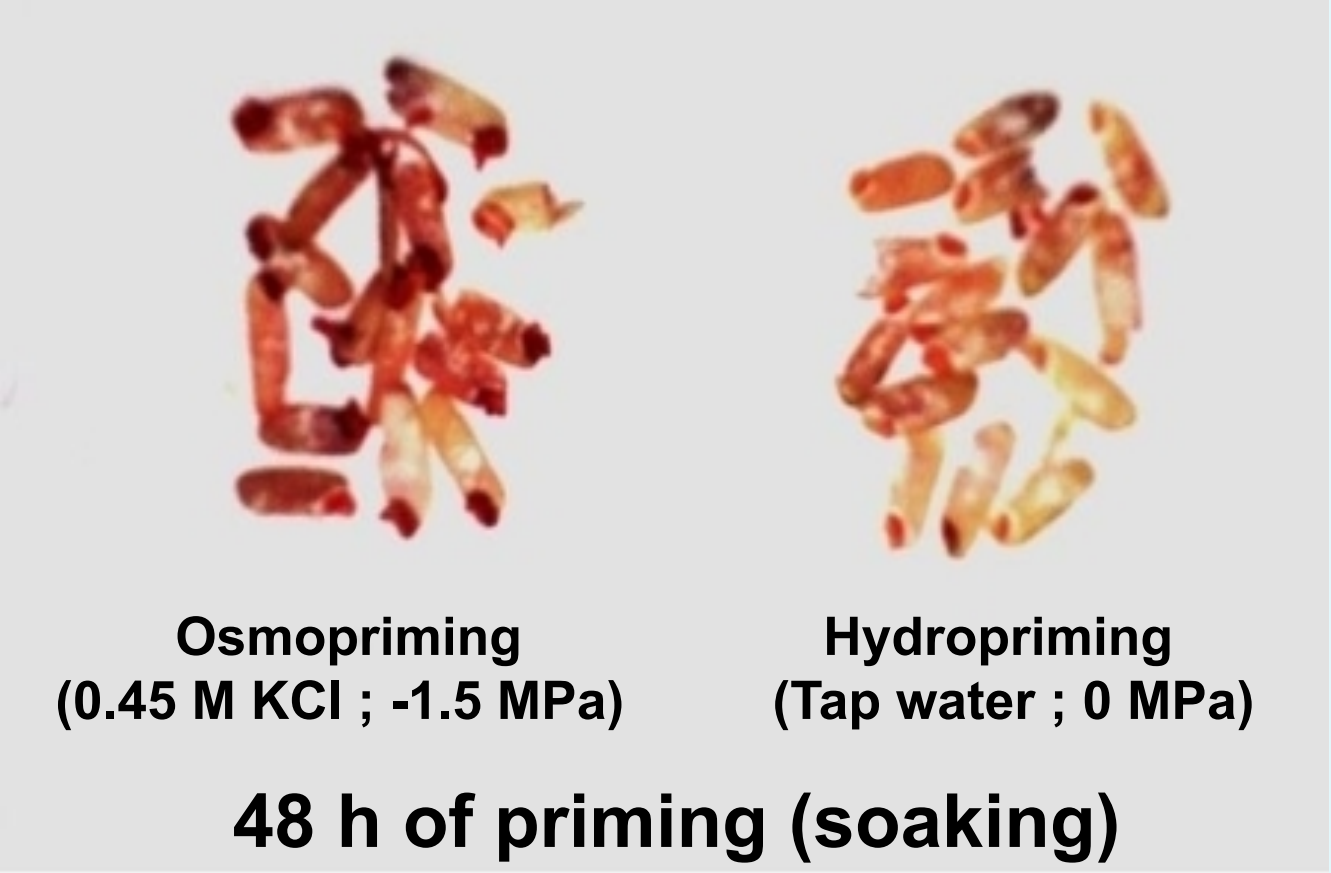

- **Hydropriming generally increased shoot and root lengths.**
  - **Tolerant genotypes always showing better growth.**




- Hydro- or osmopriming for 24h improved seedling survival
  - Higher effect in flooded condition
  - Greater effect in tolerant genotypes

- Hydropriming of longer duration (48h) had negative effects
  - In both conditions
  - In all genotypes

# Embryo viability staining 2d after seeding

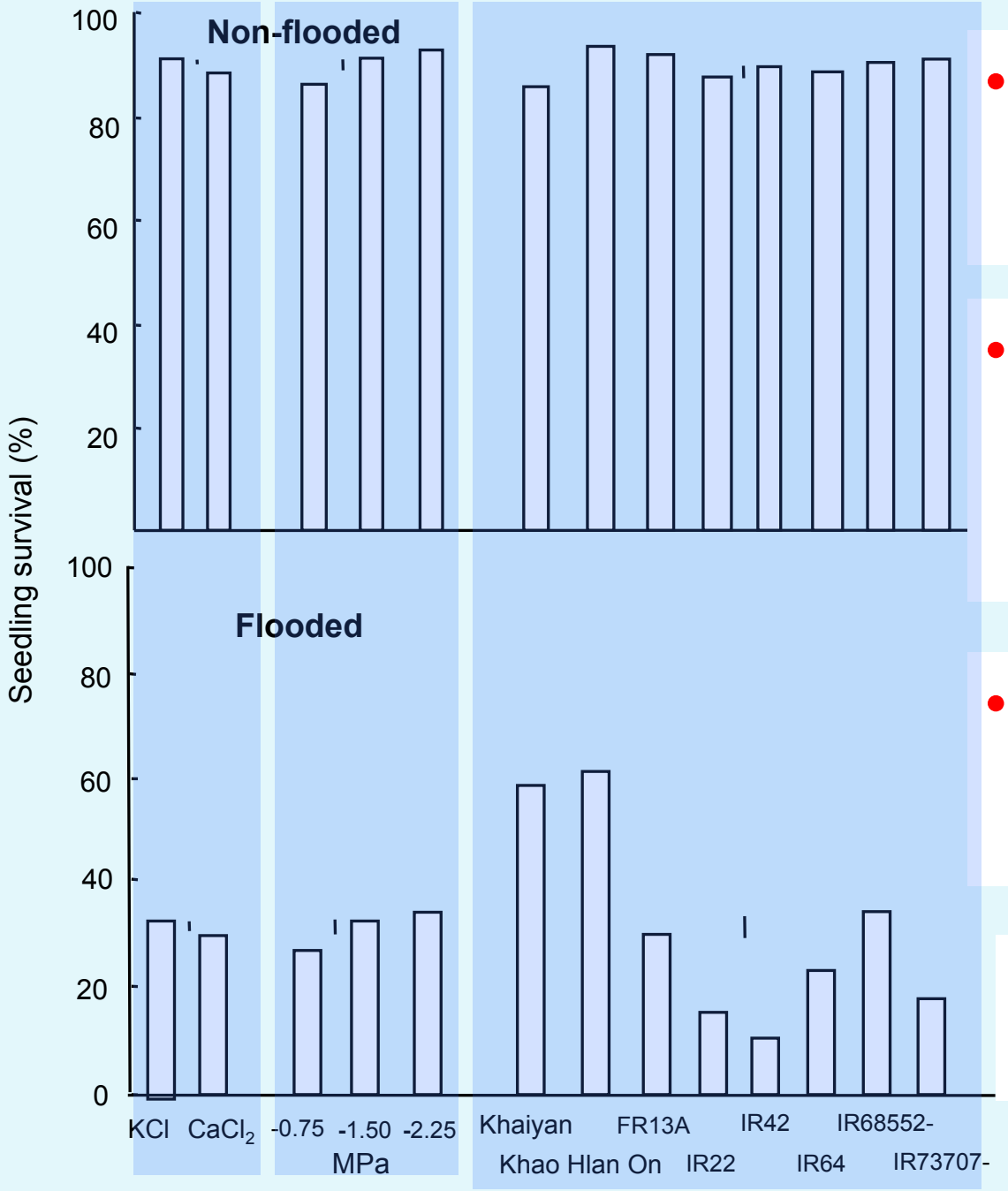


**Osmopriming**
**(0.45 M KCl ; -1.5 MPa)**

**Hydropriming**
**(Tap water ; 0 MPa)**

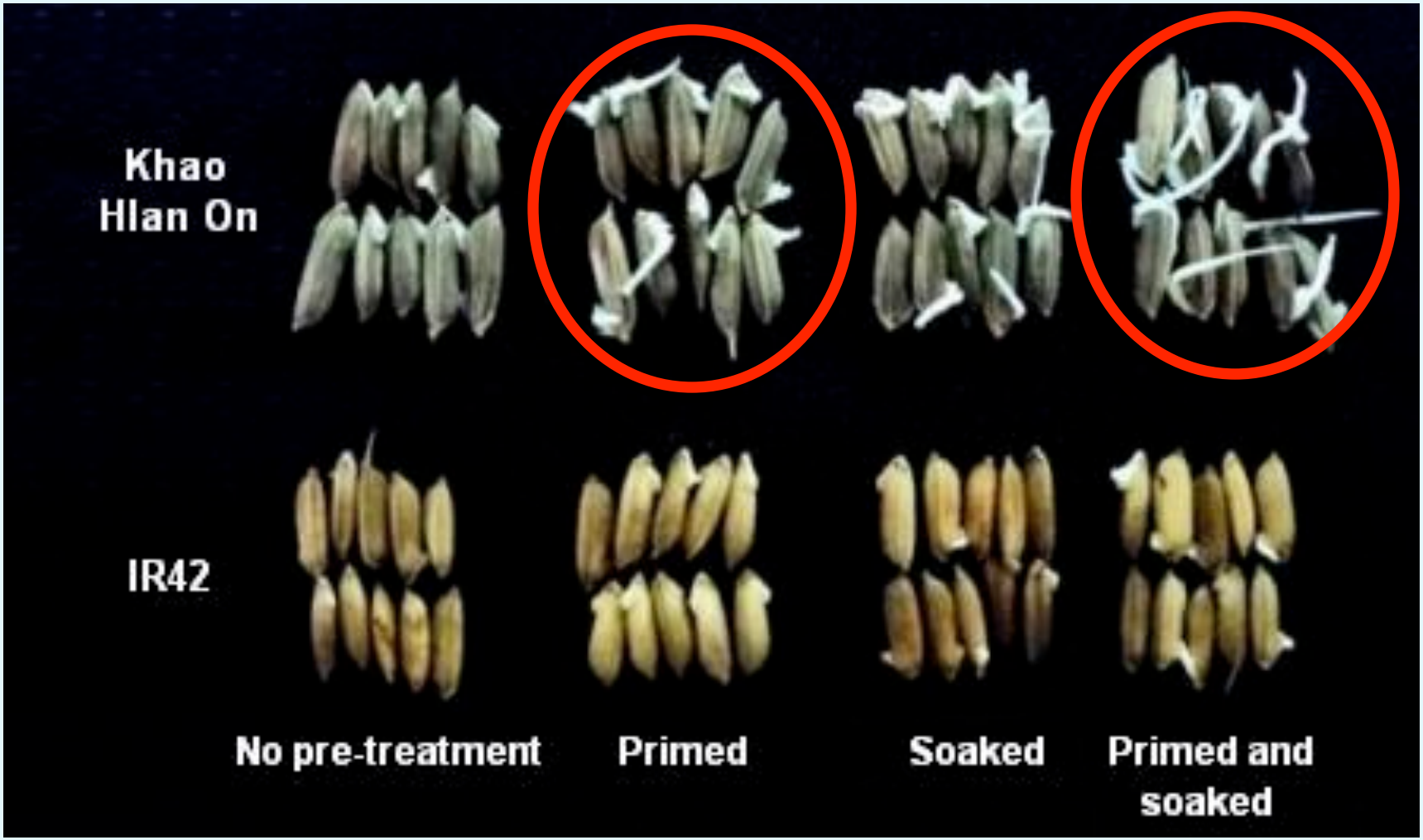
**48 h of priming (soaking)**

- **Hydropriming for 48h decreased embryo viability**



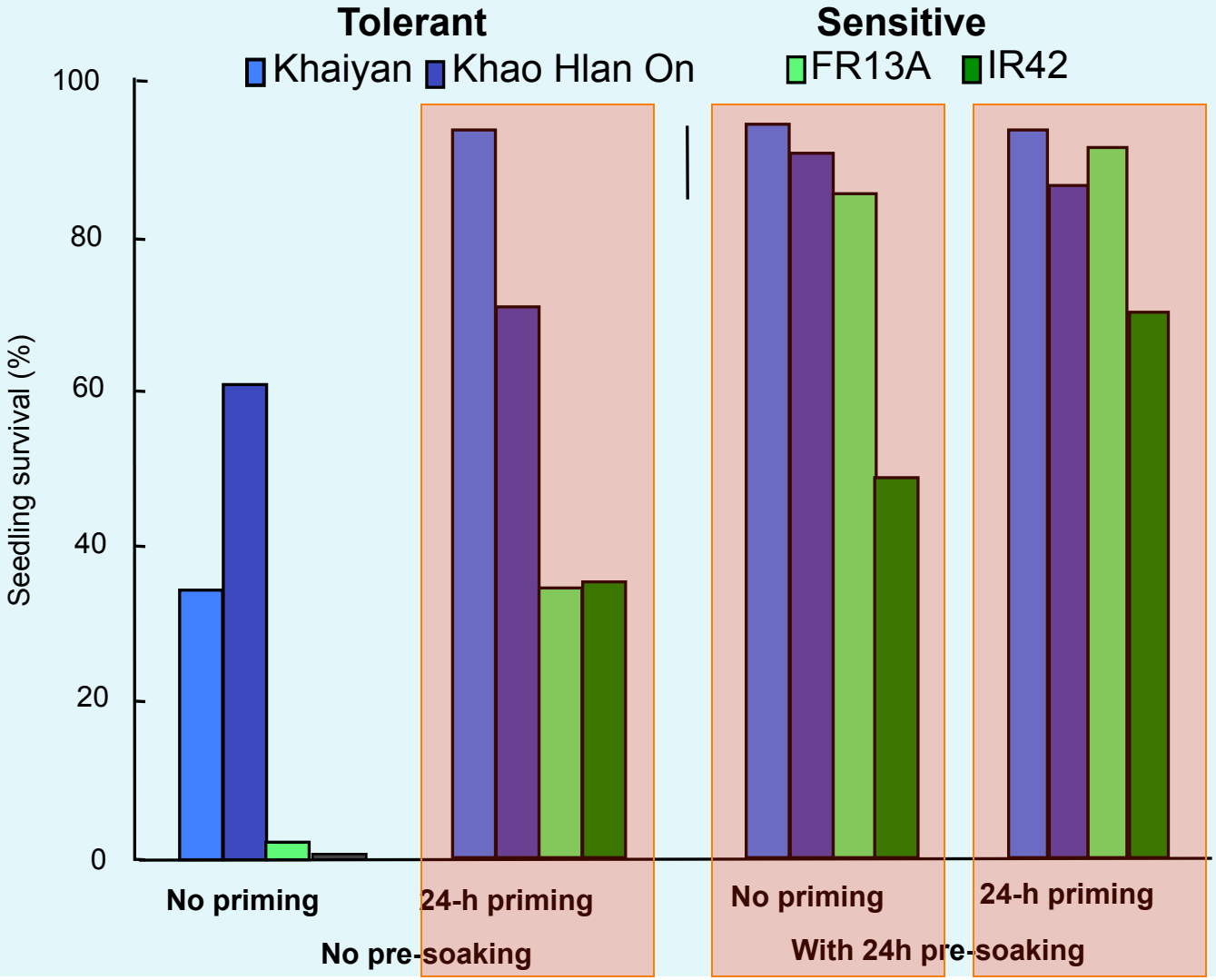

- Higher survival in seeds primed with KCl than $CaCl_2$
- Higher survival in priming of salt solution with higher osmotic potential (-2.25 MPa)
- Better priming results and earlier emergence in tolerant genotypes
- Priming effect more evident in flooded soil

# Effect of Priming and Pre-soaking on Germination

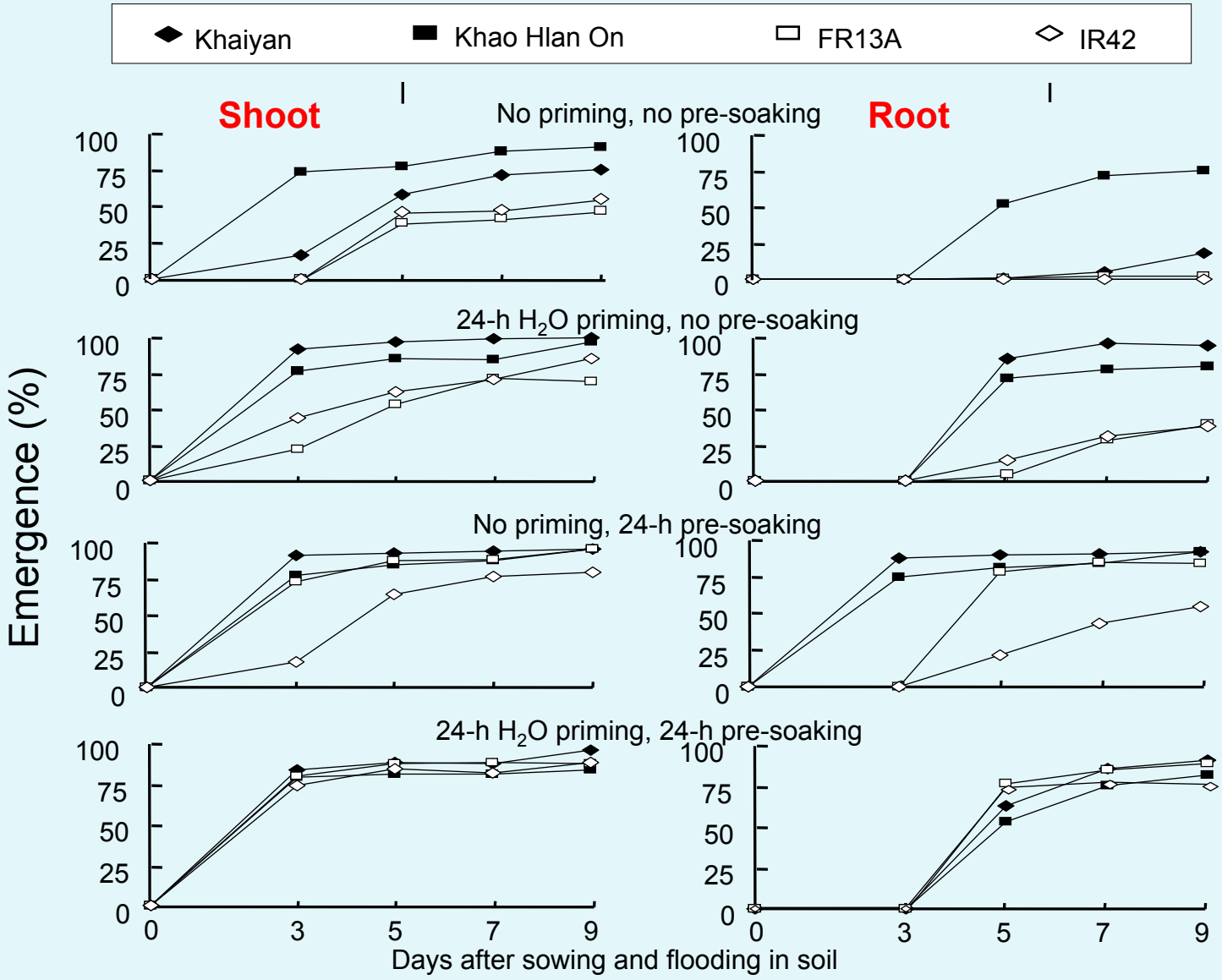



- **Hydropriming and pre-soaking before sowing resulted in earlier and more uniform germination**

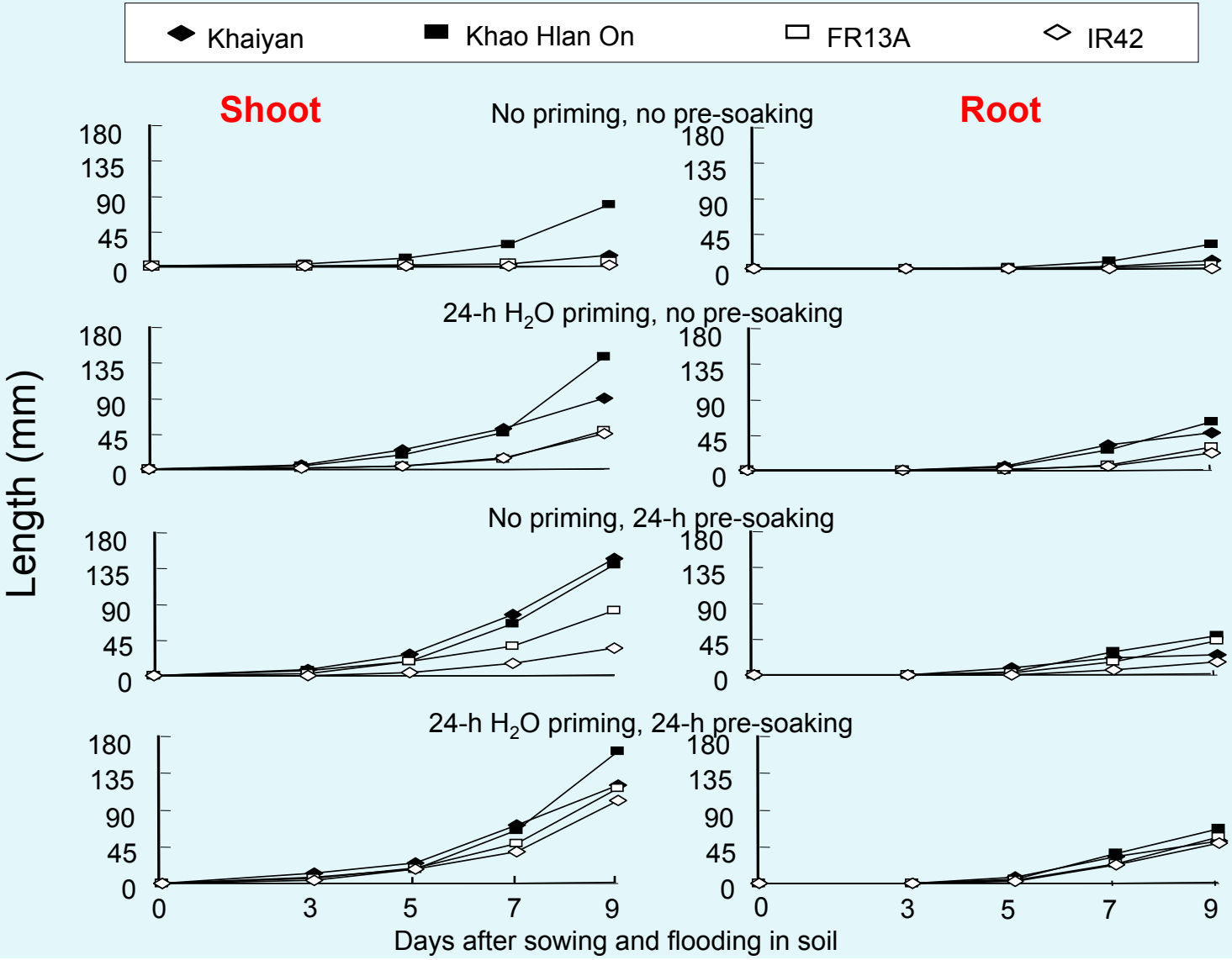
# Effect of Priming and Pre-soaking on Survival in Flooded Soil




- **Combining hydropriming and pre-soaking before sowing resulted in better survival for sensitive genotype**

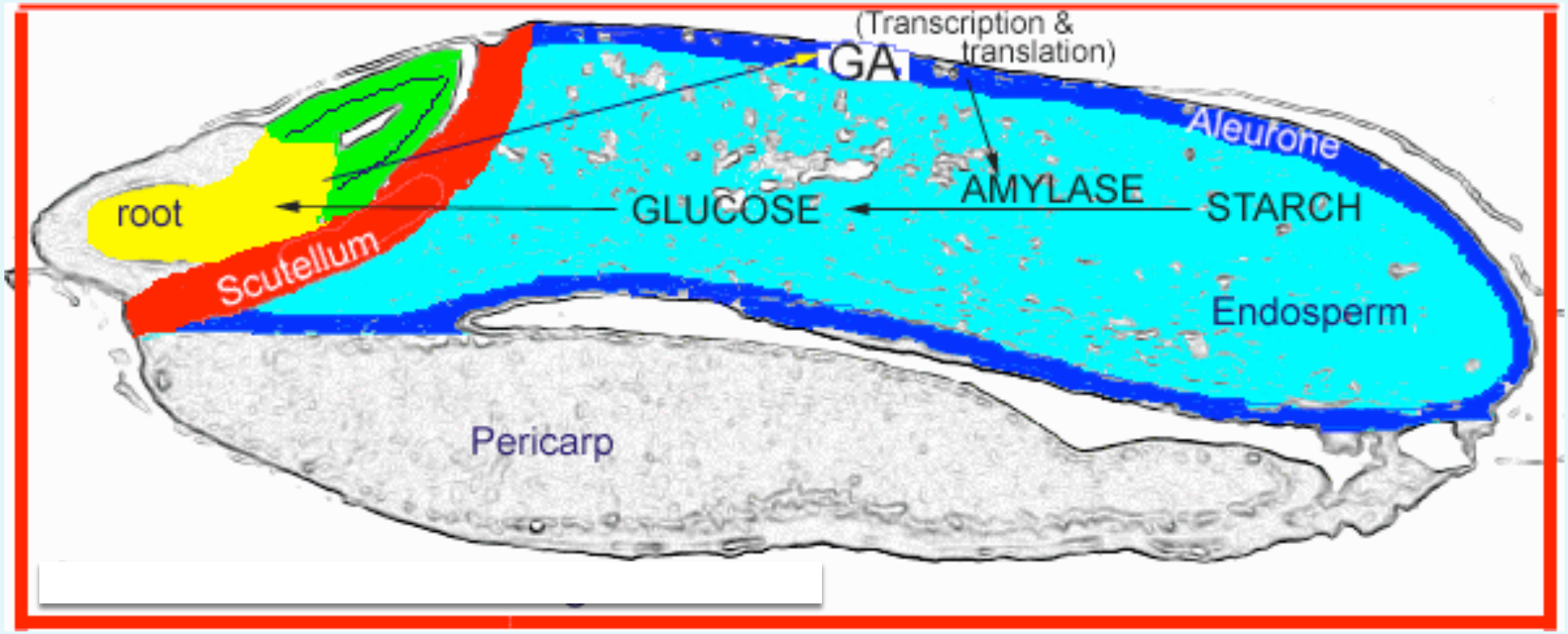



- **Hydropriming and pre-soaking before sowing resulted in enhanced shoot and root emergence**



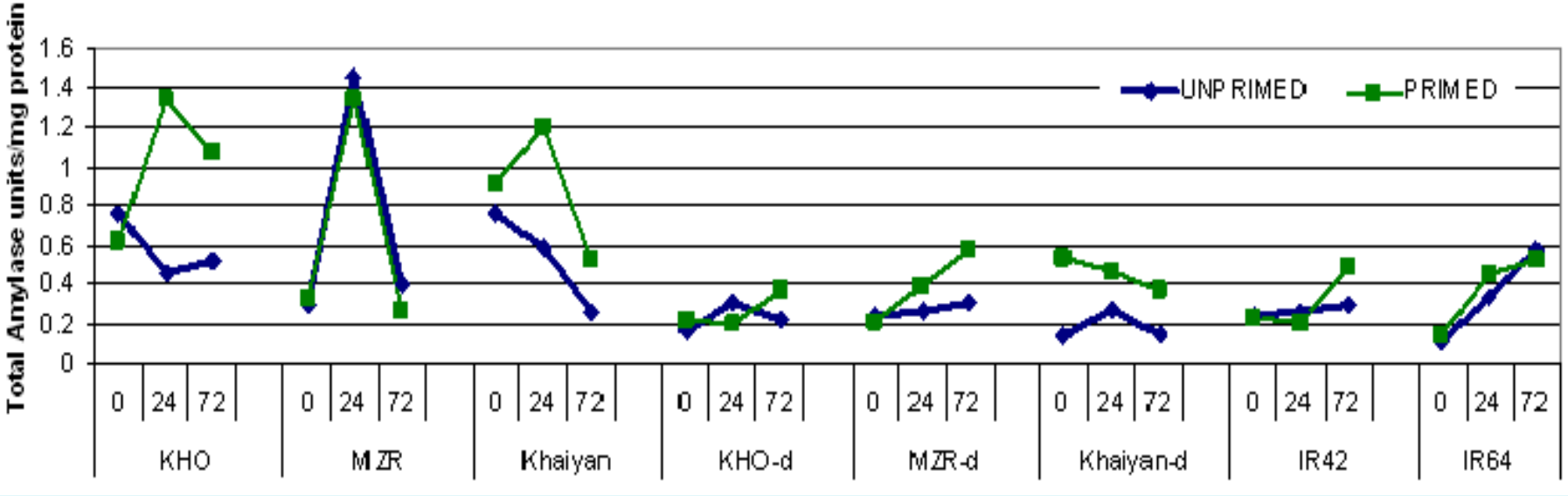

- **Hydropriming and pre-soaking before sowing resulted in longer shoot and root growth**

# Seed Carbohydrate Mobilization

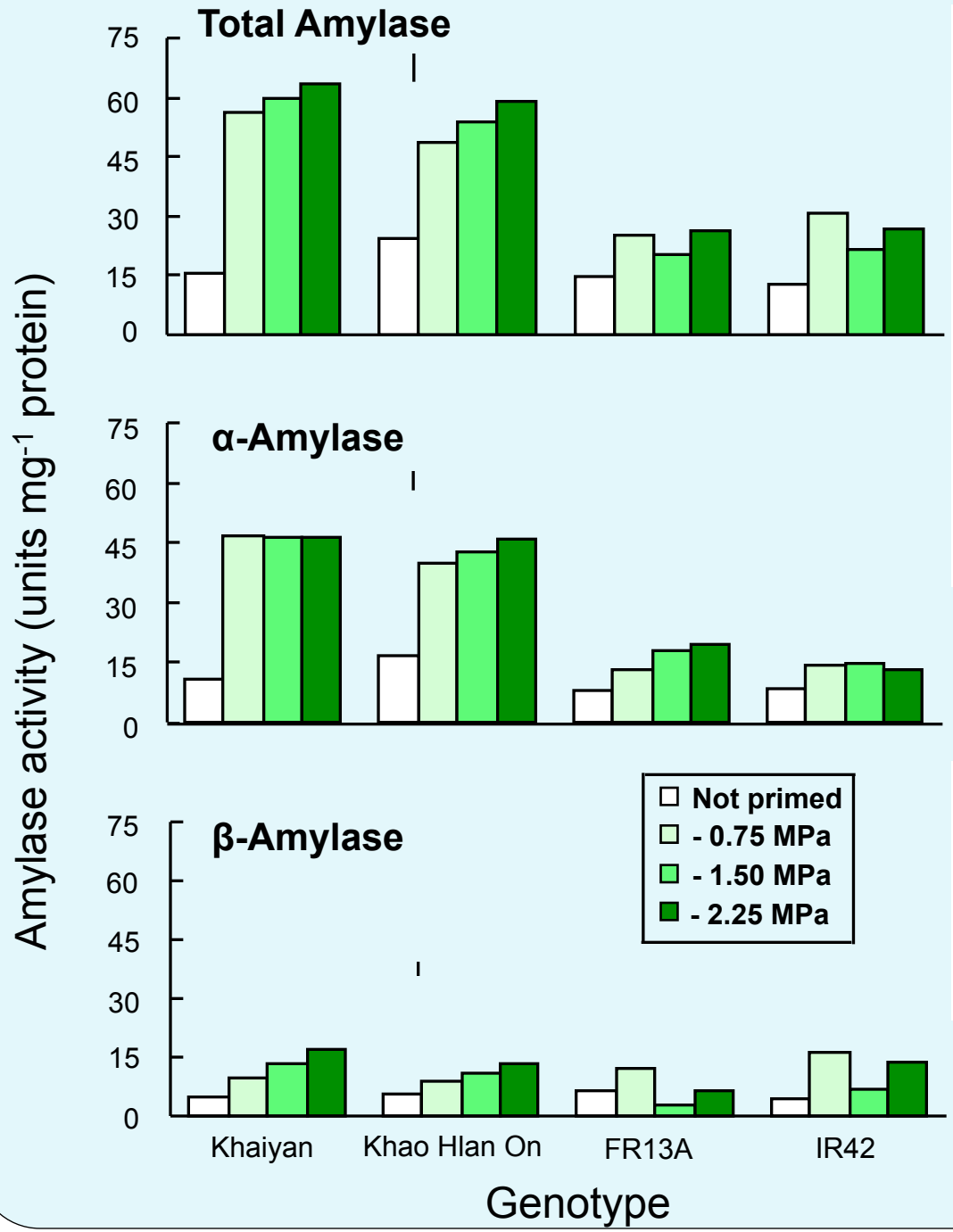



http://mol-biol4masters.masters.grkraj.org/html/Gene_Expression_II6-Plants_More_Promoter_Elements_files/image043.gif

# Hydropriming Effect on Total Amylase Activity in Hypoxic Condition



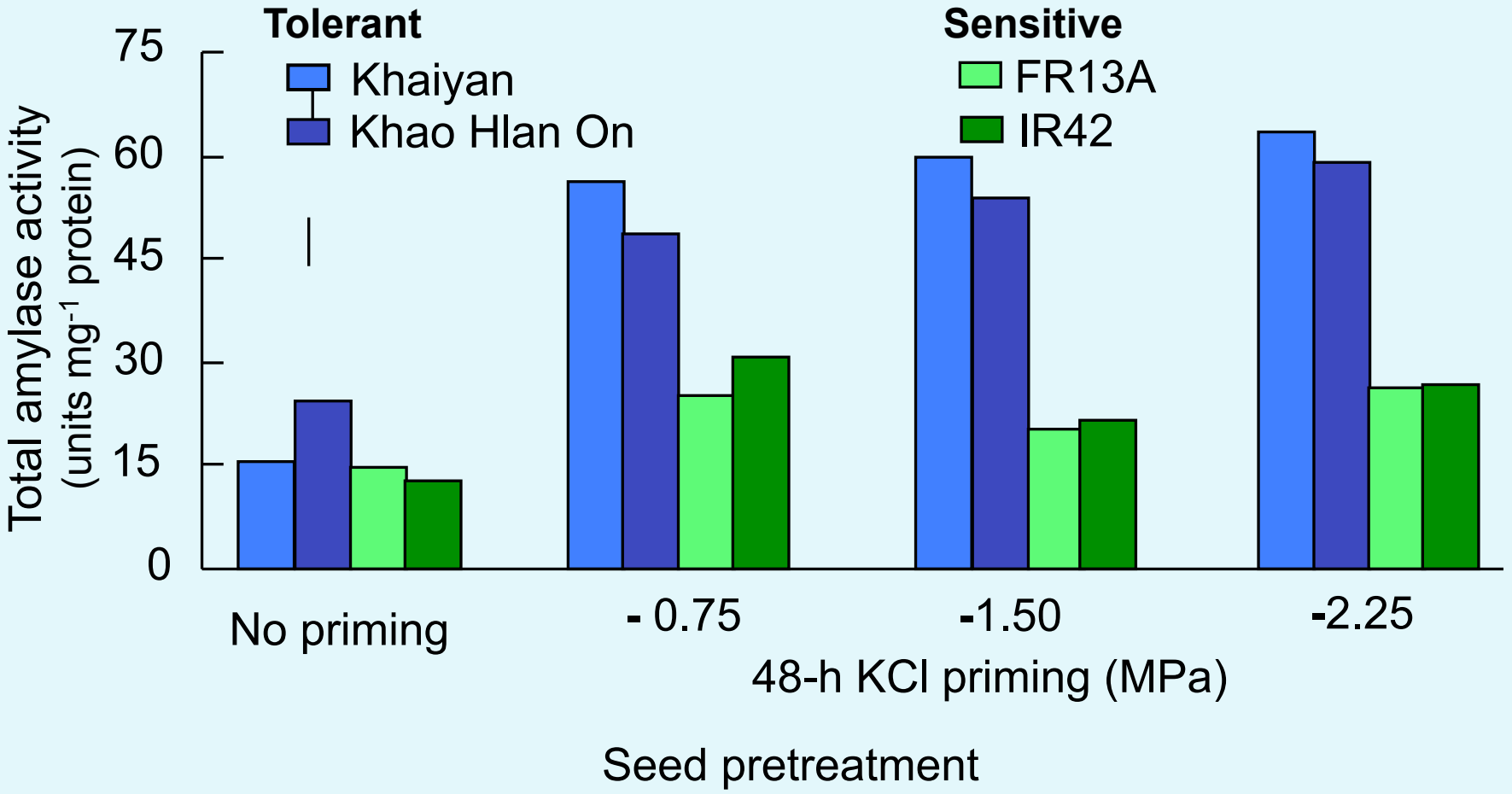

- **Hydropriming generally resulted in higher amylase activity especially in tolerant genotypes**




- Osmopriming also resulted in higher total amylase activity in flooded soil (1 day after sowing) especially in tolerant genotypes

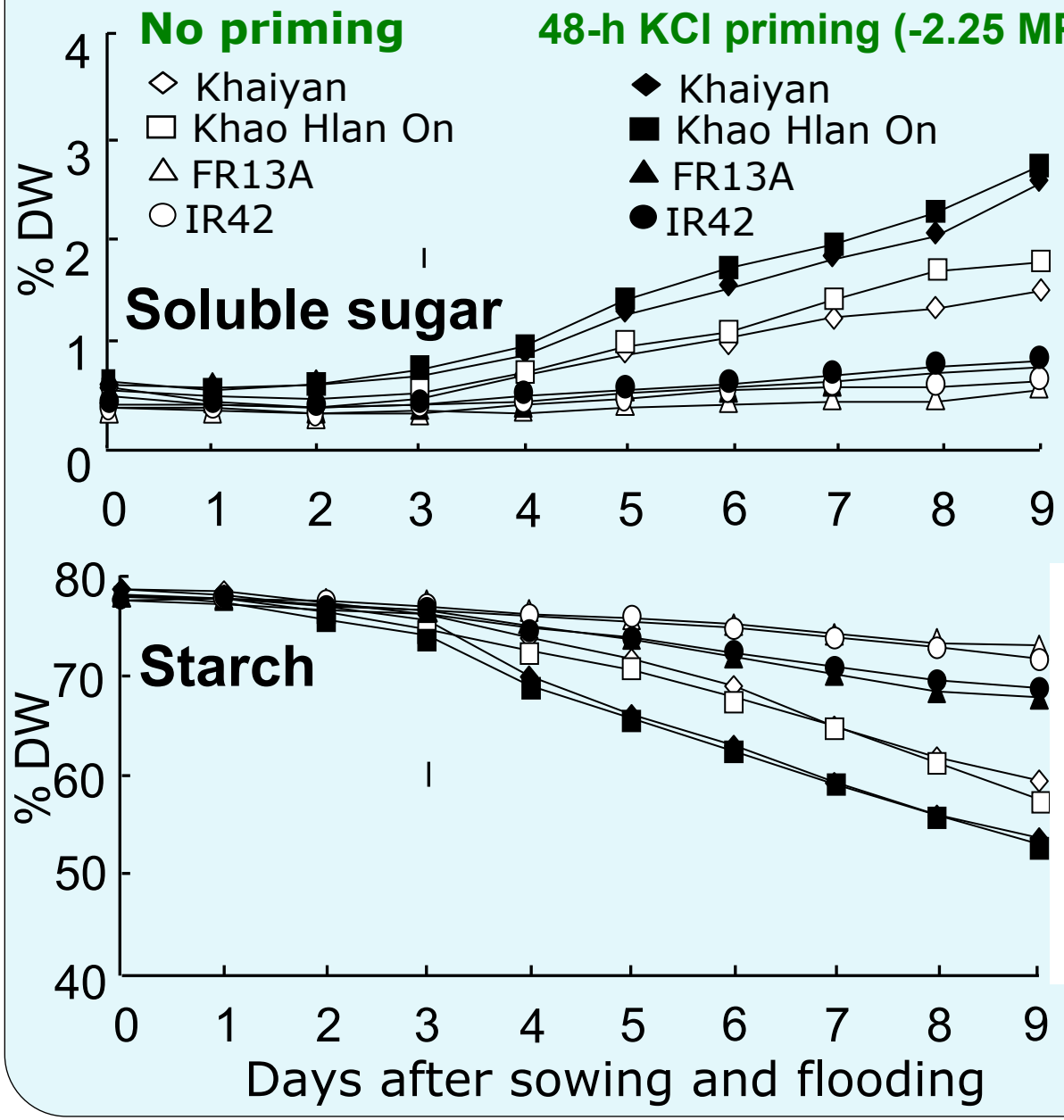
- Total amylase activity is attributed more to $\alpha$-amylase activity

# Osmopriming Effect on Total Amylase Activity in Flooded Soil (3d after sowing)




- **Osmopriming also resulted in higher total amylase activity in flooded soil 3 days after sowing**

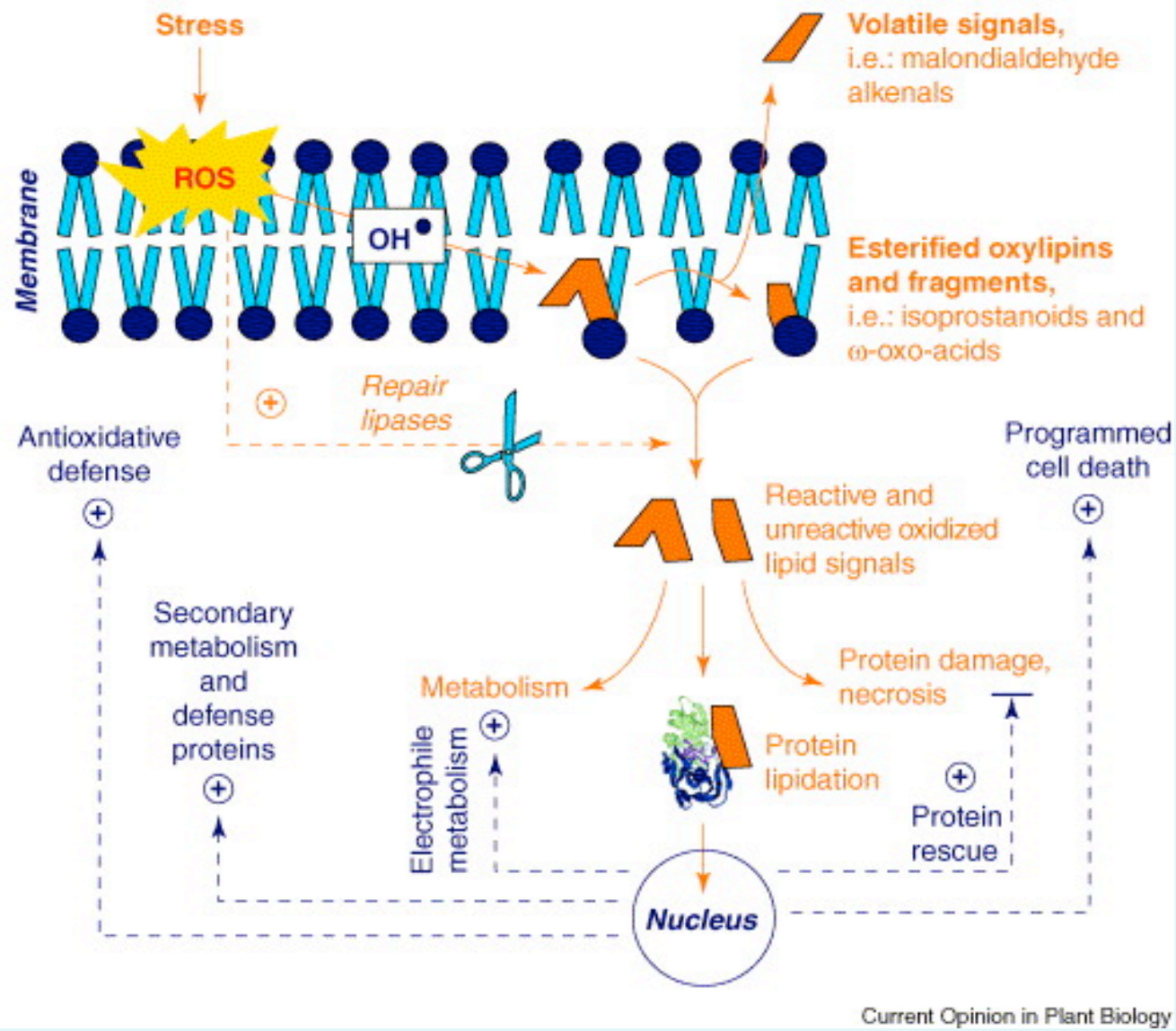
# Osmopriming Effect on Soluble Sugar and Starch




- Sugar increased while starch decreased upon sowing

- Faster mobilization of starch reserves in tolerant genotypes and upon priming

# Membrane Lipid Peroxidation

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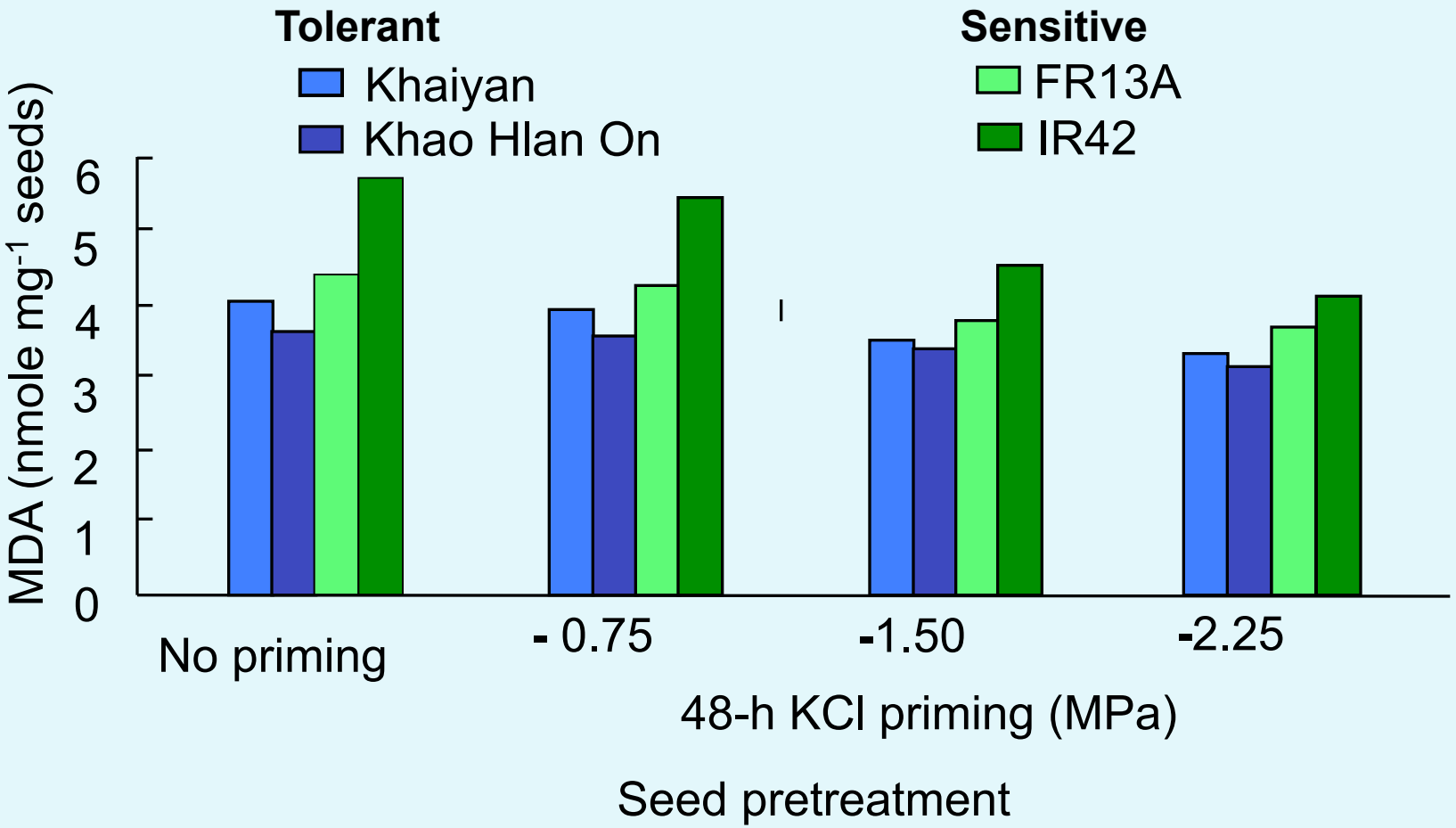
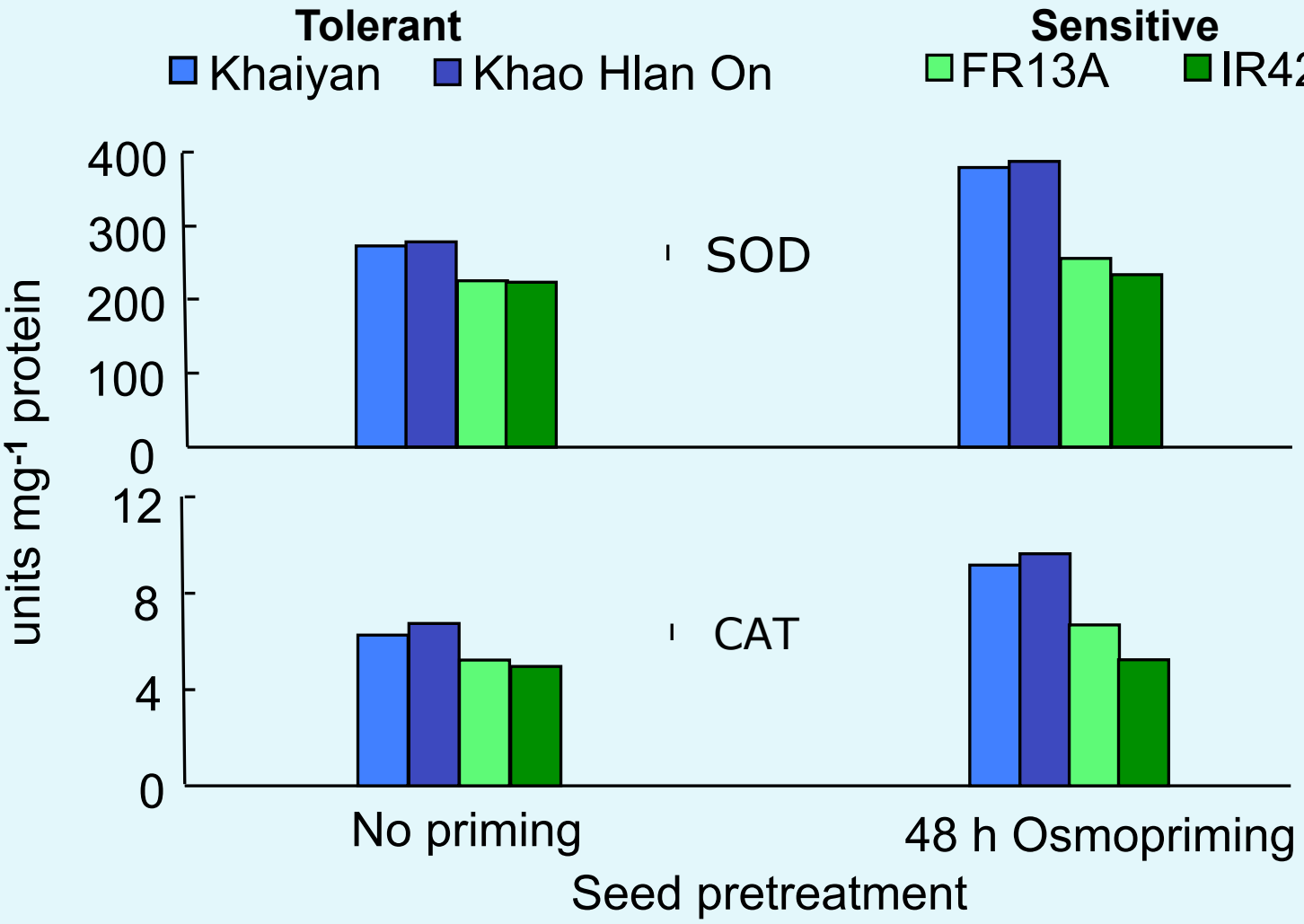
http://ars.els-cdn.com/content/image/1-s2.0-S1369526604000573-gr2.jpg

# Osmopriming Effect on Seed Lipid Peroxidation




- **Osmopriming reduced the extent of lipid peroxidation, thus decreasing seed damage**

# Osmopriming Effect on Activities of Antioxidant Enzymes




- **Increased activities of superoxide dismutase (SOD) and catalase (CAT) in osmoprimed seeds especially in tolerant genotypes**

# Osmopriming Effects on Rice Subjected to Drought

| Cultivar (Reference) | Priming Treatment | Soil Moisture Condition (SMC) | Results |
|---|---|---|---|
| Ilpumbyeo (Lee et al. 1998) | PEG (0.6 MPa) 4d then air-dry | 60, 80, 100,120, 140% FC (tray condition) | Higher (5-34%) germination and emergence rates in primed seeds<br>Priming effect greater at too lower or higher SMC |

| Cultivar (Reference) | Priming Treatment | Soil Moisture Condition (SMC) | Results |
|---|---|---|---|
| OMCS 94 (Du and Truong 2002) | KCl (14%) or sat $CaHPO_4$, 15h then sun-dry | Very dry soil, near PWP (field condition) | Primed seeds showed:<br>enhanced emergence<br>increased plant density<br>greater tiller numbers<br>higher grain yield |

# Priming Effects on Rice Subjected to Drought

| Cultivar (Reference) | Priming Treatment | Water Stress Condition | Results |
|---|---|---|---|
| *Indica*<br>Gangyou 527 (hybrid)<br>Yangdao 6 (conventional)<br>*Japonica*<br>Nongken 57 (lowland)<br>Zhonghan 3 (upland)<br>(Sun et al. 2010) | Water 24h then oven-dry<br>PEG (5%-25%) 12h then oven-dry | PEG (0, 5%, 10% 15% and 20%) 10d (petri dish) | Hydropriming of all cultivars and 15-20% PEG-primed Gangyou 527 and Nongken 57 cultivars showed higher germination vigor lower MDA and proline, higher PAL, SOD, POX, CAT act, lower soluble sugar, and higher soluble protein in seedlings<br>Promotive effects higher in *indica* than *japonica* cultivars hybrid than conventional cultivars upland than in lowland cultivar<br>PEG-priming with moderate conc resulted in higher tolerance to drought stress than hydropriming |

# Osmopriming Effect on Rice Subjected to Salt Stress

| Cultivar (Reference) | Priming Treatment | Salt Stress Condition | Results |
|---|---|---|---|
| China rice (He et al. 2002) | Mixed-salt solution | 0.58% NaCl | Primed seeds exhibited more rapid germination<br>increase in seed α- and β-amylase activities<br>increase in root dehydrogenase<br>moderate rise in shoot catalase |

# Conclusions and Recommendations

- Seed priming effective even under adverse soil conditions
- Faster germination and enhanced seedling establishment upon priming due to
  - higher activity of antioxidant enzymes
  - higher activity of amylase enzymes
- Priming using tolerant cultivars recommended

# Acknowledgements


- **Mr. Ryan John P. Pascual**
  [BS Biology (Plant Biology), UPLB *cl* 2010]
- **Dr. Evangeline S. Ella**
  [PhD by Research (Botany), UPLB 2011]
- **Dr. Abdelbagi M. Ismail**
  (IRRI Plant Physiologist)
- Mr. Mark Anthony F. Rabena
  [BS Biology (Plant Biology), UPLB *cl* 2008)
- Ms. Marianita N. Eroy
  [MS Botany, UPLB 2005]

Thank you for your attention!