Effect of seed priming on physiological and biochemical performance of rice (*Oryza sativa* L.) seedlings

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ABSTRACT: The decline in rice production in Côte d'Ivoire in recent years is partly due to the poor condition of seeds at the time of planting. In this context of climate change, it is important to explore other alternatives for higher production. The aim of this study is to understand the physiological mechanisms involved in the growth of rice plants from primed seeds. Seeds of rice variety C26 (Oryza sativa) were primed in different solutions and grown under rainfed and rainfed lowland conditions. Treatments included control, water priming, sucrose priming and NaCl priming. Seeds primed with water and sucrose favored rice plant growth. The number of leaves under these conditions varied from 20 to 53. Plant height and number of tillers varied from 15 to 45 cm and from 5 to 12 tillers respectively. Seeds treated with NaCl showed the lowest values. Photosynthetic pigment levels were highest in plants growing under rain-fed conditions between the 4th and 7th weeks after planting. As for phenolic compound content, it was higher in growing plants in the lowland system and lower in rainfed conditions. Rice seed priming improved plant growth. The C26 rice variety is better adapted to lowland conditions.

Keywords: Seed priming, Oryza sativa, growth, cropping system, biochemical compounds.

1 INTRODUCTION

Rice (Oryza sativa L.) is the most widely grown cereal in the world after wheat, accounting for 22% of global cereal consumption FAO (2020). At regional level, Côte d'Ivoire is the third largest paddy producer in the ECOWAS region, after Nigeria and Mali. Rice cultivation, which accounts for 57% of the area sown to cereals, provides 26% of the country's total food production and contributes around 17% of total agricultural employment. Rice production in 2018 was estimated at 1.3 million tonnes (Ouédraogo et al., 2021). This production is achieved through three types of rice production systems (rainfed upland rice, rainfed lowland rice and irrigated rice). Upland and lowland rice production is mainly rain-fed, with average productivity of 1.2 t/ha for upland and 2.5 to 3 t/ha for lowland. Rice consumption in Côte d'Ivoire continues to increase by at least 3% each year, with a need for around 1.8 million tonnes of rice per year (ADERIZ, 2020). At present, the country imports almost 50% of its rice consumption needs from Asia, the cost of which continues to rise. To offset this deficit, the Ivorian government has set up a structure to revitalize the rice industry (APRAO, 2012). But despite all these efforts, the country's rice deficit remains a recurring problem. One of the constraints of this production deficit is the use by producers of cultivation techniques that may or may not be adapted. Lage (1997) has described the constraints on rainfed rice production as climatic (scarcity, irregularity and inadequacy of rainfall), parasitic (prevalence of blast and helminthosporiosis), edaphic (decline in soil chemical fertility) and varietal (low-productivity cultivars).

For irrigated lowland and rainfed lowland rice, the main constraints are due to lack of control over irrigation water, yellow mottle, blast and iron toxicity. In order to help improve the germination performance, development and yield of plant species, as well as their tolerance to abiotic stresses, the priming technique has generally been used. It accelerates and synchronizes germination (Bradford, 1986; Taylor and Harman, 1990; McDonald, 2000). It also promotes better growth, earlier flowering, greater tolerance to stress and higher yields (Harris et al., 2002; Basra et al., 2006; Moosavi et al., 2009). In this context of climate change, it is important to gather information on the growth of plants derived from grains that have undergone priming, in order to initiate suitable approaches. With this in mind, this study was undertaken to understand the physiological mechanisms involved in the growth of rice plants following the priming technique.

2 MATERIALS AND METHODS

2.1 PLANT MATERIAL

The plant material used in this experiment consisted of rice seeds of variety C26. They were supplied by AMC-FC (Agricultural and Mangement Company Food and Commerce) based in Yamoussoukro (Côte d'Ivoire). This variety is characterized by its 50% semi-maturity cycle, which takes 102 days. It is tolerant to lodging and drought, with a weak aroma and long grain type (CORAF, 2018). It is adapted to rainfed and lowland conditions and has a potential yield of 7.5 t/h.

2.2 METHODS

2.2.1 EXPERIMENTAL SET-UP

The experimental set-up used in this study was a factorial block design, consisting of three blocks and two factors. This setup consisted of twenty-four elementary plots containing the treatments.

Each block took into account the cropping system factor (rainfed uplands and lowlands) and the pregerminative treatment (priming), which comprises four modalities (Control, water priming, sucrose priming and NaCl priming). The eight defined treatments were established randomly on the elementary plots of each block. Each elementary plot was 3 m long and 2 m wide. Each block consisted of two rows of elementary plots. The elementary plots in a block were separated by 1 m on the line and by 2 m on the two lines. The blocks were spaced 3 m apart and 1 m apart with the border. Seeding points were separated by 20 cm on the row and between rows. Plant density on an elementary plot was 126 plants. The total surface area of the plot was 408 m².

2.2.2 DIFFERENT TREATMENTS AND CULTIVATION METHODS

Low-lying areas were created so that all plots were under the same experimental conditions. Water was added on a permanent basis. After a flotation test to identify viable seeds, they were divided into four 0.5 kg batches. The first batch (T0) was the Control and did not undergo priming. The second batch (T1) underwent 43 hours of priming in water. Seeds were soaked in 1 liter of distilled water. The third and fourth batches of seeds were soaked in 1 liter solutions of NaCl (1 mol/l) and sucrose (0.18 mol/l). The seeds were soaked for 43 h, then dehydrated by drying in the open air but in the shade in a room for two days. Room conditions were $27 \pm 2^{\circ}$ C with a relative humidity of 70-80%. Seeds were weighed regularly until they reached a constant weight, close to their initial weight. The seeds were stored for two weeks and used as seed.

Direct sowing was carried out at a depth of 3 cm.

PARAMETER EVALUATION

Evaluation began in the fourth week after sowing and ended in the ninth week after sowing. These were the number of leaves (NF) emitted by hand-counting, plant height using a decameter, number of tillers per bunch by hand-counting, average leaf length of expanded leaves of rows 3, 4 and 5 using the same decameter, total chlorophyll and carotenoid content of according to the formulas of Lichtenthaler and Buschmann (2001) and phenolic compound content (Kouakou, 2009).

2.2.3 STATISTICAL ANALYSIS

The data collected during the experiments were analyzed using STATISTICA version 7.1 software. The conditions for carrying out the analysis of variance were verified. The evaluation for each treatment was carried out on 36 plants. The homogeneity of variance was then tested using Bartlett's test with a threshold of 5%. Two-factor analysis of variance (ANOVA) (type of rice

cultivation and type of priming) was adopted to assess the influence of the different treatments on morphological and biochemical parameters. In the absence of interaction between the two factors, an ANOVA 1 was performed for the main effects. When this analysis showed a difference between the means of the factors at the 5% threshold, Tukey's test at the 5% threshold was performed to classify the means.

3 RESULTS AND DISCUSSION

3.1 RESULTS

3.1.1 COMBINED EFFECT OF PRIMING AND CROPPING SYSTEMS ON RICE PLANT GROWTH

Number of leaves

Statistical analysis of the data revealed an interaction between priming and cropping system (P < 0.001) on the number of leaves in all weeks. Plants from seeds primed with water (H2O) and sucrose in the lowland system induced more leaves, ranging from 20 to 55, from weeks 4 to 9. In contrast, the other treatments induced fewer leaves, ranging from 13 to 38 (Figure 1).



Fig. 1. Foliar emission of rice plants grown from seeds initiated during growth under rainfed upland and rainfed lowland conditions

Comparison carried out at each time between treatments. $P_{interaction}$: ($P_{Week 4} < 0,001$); ($P_{Week 5} < 0,001$); ($P_{Week 6} < 0,001$); ($P_{Week 7} < 0,001$); ($P_{Week 7} < 0,001$); ($P_{Week 7} < 0,001$); ($P_{Week 9} < 0,001$); (

LEAF LENGTH

Figure 2 shows that plants growing in the lowland system produced the longest leaves for all primed seeds, particularly in water, where leaf length reached around 50 cm by week 9. Plants from the rainfed upland system showed short leaf lengths for all primed seeds. NaCl priming and the growing control in the rainfed tray system induced short lengths that ranged from 20 cm to 37 cm.



Fig. 2. Evolution of leaf length during the growth of rice plants from different treatments in rainfed and lowland conditions

Comparison carried out at each time between treatments. $P_{interaction}$: ($P_{week 4} < 0.001$); ($P_{week 5} < 0.001$); ($P_{week 6} < 0.001$); ($P_{week 7} < 0.001$); (

PLANT HEIGHT

Statistical analysis of the data revealed no interaction between growing system and priming (Pinteraction > 0.05). However, a cropping system effect and a priming effect were observed for plant height (P < 0.001). Thus, the rainfed lowland system favored better rice seedling growth than the rainfed upland system (Figure 3).

Seeds primed with NaCl induced the lowest heights, ranging from 12 to 19 cm. In contrast, priming with water and sucrose induced the highest plant heights, ranging from 13 to 45 cm (Figure 4).



Fig. 3. Height of rice plants in rainfed and lowland conditions during growth

Comparison between treatments at each stage. $P_{significatif}$ ($P_{semaine 4} < 0,001$); ($P_{week 5} < 0,001$); ($P_{weed 6} < 0,001$); ($P_{week 7} < 0,001$); ($P_{week 8} < 0,001$); ($P_{week 9} < 0,001$); (P_{week



Fig. 4. Evolution of plant height from primed seeds during growth

Comparison made at each stage between treatments. $P_{interaction}$: ($P_{week 4} = 0,0023$); ($P_{week 5} = 0,003$); ($P_{week 6} = 0,001$); ($P_{week 7} < 0,001$); ($P_{week 8} < 0,001$); ($P_{week 9} < 0,001$); (P_{we

NUMBER OF TILLERS

The evolution of the number of tillers is shown in figure 5. Statistical analysis of the data revealed an interaction between seed priming and cropping system over the 9 weeks (P < 0.001). In fact, seed priming with water and sucrose favored tillering of growing plants in the rainfed lowland. The number of tillers varied from 4 to 12 from week 4 to week 9. However, seedlings

from NaCl-primed seeds and those from the control in the rainfed upland system showed a lower number of tillers, ranging from 3 to 8.



Fig. 5. Tallage of rice plants from different treatments in rainfed and lowland conditions

Comparisons made between treatments at each stage. $P_{interaction}$: ($P_{week 4} < 0,001$); ($P_{week 5} < 0,001$); ($P_{week 6} < 0,001$); ($P_{week 7} < 0,001$); ($P_{week 8} < 0,001$); ($P_{week 9} < 0,001$); (P_{we

3.1.2 COMBINED EFFECT OF PRIMING AND CROPPING SYSTEMS ON TOTAL CHLOROPHYLL (CHLT) AND CAROTENOID CONTENT OF RICE LEAVES

TOTAL CHLOROPHYLL CONTENT

Data analysis revealed a cropping system x seed priming interaction effect (P < 0.001). In general, chlorophyll content was higher in plants from seeds primed with water, NaCl and sucrose growing in the rainfed upland system from week 4 to week 7. However, carotenoid content was higher in plants from the rainfed lowland system from weeks 8 to 9 (Figure 6).

CAROTENOID CONTENT

Carotenoid content is shown in Figure 7. Statistical analysis of the data revealed a priming type x cropping system interaction effect (P < 0.001). Carotenoid content was high in all plants except those primed with water (H2O), from weeks 4 to 7. This content was lower from week 8 onwards. At the start of the seedling cycle, carotenoid content was very high in both growing systems in all seedlings grown from primed seeds. However, from week 8 onwards, at the start of fruit set, carotenoid levels dropped significantly in all plants.

3.1.3 EFFECT OF PRIMING ON THE PHENOLIC CONTENT OF RICE SEEDLINGS

The evolution of phenolic compound content in seedlings from primed seeds is shown in Figure 8. Statistical analysis showed that there was a priming type x growing system interaction effect (P < 0.001).

Phenolic content decreased from week 4 to week 9 in both control and NaCl-primed seedlings. However, a gradual increase in phenolic compound content was observed in plants from the lowland system, with seeds primed with water and sucrose respectively.





Histograms marked with the same letter are statistically identical at the 5% threshold - Tukey's test (mean ± standard error).



Fig. 7. Carotenoid content of rice plants from treatments during growth in rainfed and lowland conditions

Histograms marked with the same letter are statistically identical at the 5% threshold - Tukey's test (mean ± standard error).





Histograms surmounted by the same letter are statistically identical at the 5% threshold -Test of Tukey (Mean ± standard error)

3.2 DISCUSSION

The results of the study revealed the influence of priming and cropping systems on the morpho-physiological parameters assessed. NaCl priming induced delayed growth in rice plants in terms of height and number of leaves in both cropping systems. This growth delay in the NaCl-primed plants could be explained by the fact that, rice being a glycophyte species, a low salt concentration would lead to a drop in cell turgor pressure, resulting in poor cell division and expansion. This, in turn, slows plant height growth and the number of leaves. The low growth rates favored by NaCl priming can be explained by the fact that it reduces cell division and expansion (Benmahioul et al., 2009; Sobhanian et al., 2010; Mohammed et al., 2012).

Under our growing conditions, water priming (hydropriming) induced the best growth performance in rice plants. The high growth rates induced by hydropriming could be explained by the fact that the water used for priming led to hydrolysis of endosperm tissue and stimulation of endo- β -mannanase activity, resulting in strong growth. In addition, hydropriming would have favored the breakdown of mechanical resistance for cell elongation in the embryo and embryonic coverage. Plant growth parameters were thus stimulated by water imbibition, while NaCl priming had an inhibitory effect.

The results of this study showed that the variation in chlorophyll content was a function of plant age. Compared with the control, the chlorophyll content of primed plants evolved in almost the same way. When the plant is at a younger stage (4 weeks after sowing), chlorophyll content is lower. Photosynthetic activity is highest at the young plant stage (5 to 7 weeks), and during the production phase (8 weeks) photosynthetic activity drops sharply. This could be explained by the fact that at the younger stage, the plant is in an intense vegetative phase, during which the organs are setting up and developing, hence the low chlorophyll content.

On the other hand, the young plants have reached an optimal level of development. Chlorophyll levels are at their optimum, and photosynthetic activity is intense. At the 8-9 week stage, corresponding to the last phase of the plant life cycle, photosynthetic activity is more focused on caryopsis maturation. The drop in chlorophyll content in rain-fed plants is the result of reduced stomatal opening to limit water loss through evapotranspiration and increased resistance to the entry of atmospheric CO2 required for photosynthesis (Bousba et al., 2009). The observed drop in chlorophyll content in rainfed plants is the result of the synergy of several factors: reduced stomatal opening, which limits water loss through evapotranspiration and increased resistance, and reduced entry of atmospheric CO2 required for photosynthesis. Reduced chlorophyll content

disrupts the redistribution of assimilates stored by the stem to the various parts of the plant, disrupting growth (Karima et al., 2012).

Carotenoid content rises at the beginning of the cycle, only to fall slightly after the fruiting period, while chlorophyll content declines very rapidly. These results could be explained by the fact that when the leaves reach their adult stage, they begin to supply the elements required for plant growth. The pigment apparatus controls its optical properties in the visible range, thanks to the high concentration of chlorophyll and carotenoids. Once this mature phase has passed, the leaf begins to senesce, and some of its elements are mobilized towards storage organs. Numerous studies detail the dynamics of pigments during the life of the leaf (Matile, 2000; Hörtensteiner and Feller, 2002).

The results of this study also revealed a progressive increase in the quantity of phenolic compounds during the growth of rice seedlings from water- and sucrose-primed seeds. Thus, the amount of phenolic compounds in plants from NaCl-primed seeds and the control decreased progressively from week 4 to week 9. The high level of phenolic recorded in the water-primed rice leaves could be explained by the rapid growth of the water- and sucrose-primed seedlings, compared with the delayed growth of the control and NaCl-primed plants. This indicates that phenolic compounds are involved in such varied roles as defense reactions against aggressors, cell growth, organogenic differentiation, bud dormancy, flowering, fruiting and induction of somatic embryogenesis (Bovy, 2004; Boizot and Charpentier, 2006). Seed priming induced significant quantities of phenolic compounds.

4 CONCLUSION

At the end of this study, it clearly appears that water priming (hydropriming) and sucrose priming (osmopriming) were the best pregerminative treatments. In fact, these treatments produced the best growth performance in rice plants, in terms of number of leaves, plant height, number of tillers and leaf length. Sucrose priming had almost the same effect on the plants as water priming. The lowland cropping system outperformed the rainfed system in terms of plant growth for rice variety C26. Leaf pigment content was more influenced by the cropping system than the type of priming. Seed priming resulted in intense phenolic compound activity in seedlings produced by water and sucrose priming. The physiological mechanisms involved in growth are cell division, cell expansion and cell hydrolysis.

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