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Effect of Soaking Durations and Seed Priming Agents on the Biochemical Properties of BPT 5204 Seedlings

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ABSTRACT

The present investigation was carried out at Agricultural College, Bapatla to know the effect of soaking time and seed priming agents on biochemical parameters of BPT 5204 seedlings. The experiment was laid out in a factorial Completely Randomized Design (CRD) with three replications involving soaking of seeds in various priming agents for different soaking periods (0, 6, 12, 24 and 48 hrs). The experimental results revealed that, higher starch and lower alpha amylase, total sugar content was recorded at 0 hrs priming duration whereas lower starch, higher content alpha amylase and total sugar content was recorded with 12 hrs priming duration. Of all the priming agents, soaking of seeds in GA @ 150 ppm resulted good with lower starch and higher amounts of alpha amylase, total sugars indicating favourable changes in the germinating seedlings.

Keywords: *BPT 5204, Soaking time and Seed Priming agents.*

With population upsurge, world's rice demand is projected to escalate upto 50% by 2050 (IRRI, 2020). Rice is predominantly grown by transplanting, but several problems such as high water and labour demands, have rendered it unprofitable in the current climate change scenario. In such cases, direct seeded rice is the best alternative. The transition to DSR reduces water usage, crop duration and labour requirements while also lowering negative environmental impacts. However, it is not without risks that could compromise the crop's overall agronomic performance.

Most of the farmers in the NSP Command Area have switched to direct seeded rice due to delayed release of canal water, resulting dry conditions in the field creating abiotic stress at seedling stage, which also coincides with severe weed problem hampering the seedling growth.

Seed priming is the best option through which robust and vigourous seedlings will be produced and can perform well under such hostile conditions. Seed priming is the process of controlled hydration of seeds that allows pre-germinative metabolic activities to proceed, but prevents actual emergence of the radical (Harris *et al*., 2002).

Starch metabolism, which is associated with alpha amylase is of great importance during seed germination which influences seedling vigour under stress condition. The scutellar epithelium of the embryo and aleurone layer of the endosperm are the sources of á-amylase. Alpha-amylase in aleurone layer hydrolyses the endosperm starch into sugars, which endow energy for the growth of shoots and roots (Beck and Ziegler, 1989). Trethewey and Smith (2000) reported that á-amylase serves as a major source of energy for rice embryos in germinating seeds.

Krishnasamy and Seshu (1989) reported that, rice cultivars with higher alpha-amylase activity showed higher germination rate and faster seedling growth at the early stage. The enzymes responsible for the mobilization of storage protein, carbohydrate mobilization (á and â amylases) and lipid mobilization (isocitrate lyase) were also activated. Further, recent molecular evidence indicates that, increase in seedling vigour is due to increase in de novo transcript of gibberellic acid (GA_3) biosynthesis during germination (Catusse *et al*., 2008) and the regulating effect of GA and ABA on the induction of á-amylase during seed germination (Gubler *et al*., 1995), which is directly associated with vigour of the seedlings.

Higher energy turnover and increased metabolism rate in primed seeds will finally lead to better germination and better seedling establishment. Thus, reduced seed germination time, uniform emergence, improved allometric attributes, vigorous growth, better root system with improved nutrient uptake, biomass accumulation and enhanced resource partitioning altogether can enhance the agronomic performance of the crop (Harris *et al*., 2002 and Clark *et al*., 2001).

The success of seed priming depends on several factors of which the important ones are choosing right priming agent, right concentration and soaking for right duration. With these facts in view, an attempt was made to find out the effectiveness of priming durations and different priming agents in mobilization of seed reserves.

MATERIAL AND METHODS

Present investigation was carried out at Post Graduate (P.G) Laboratory, Department of Agronomy and Central Instrumentation Cell (CIC), Agricultural College, Bapatla during *kharif*, 2021. Breeder seed of BPT 5204 variety seeds were procured from Agricultural Research Station, Bapatla. The initial seed moisture content was 10 %.

The seeds were soaked for different durations (0, 6, 12, 24 and 48 hrs) in various priming agents with standardised concentrations. The ratio between seed weight to the volume of priming solution was set as 1:5. After soaking, they were dried back to initial moisture. The seeds were placed on petriplates, moistened periodically with double distilled water and allowed for germination. Starch, alpha amylase and sugars were estimated in 7 day old seedlings.

The biochemical analysis of the samples were carried out following the standard procedures. Starch was estimated by anthrone reagent as per the procedure described by Sadasivam and Manickam (1996). Alpha amylase activity in the sample tissue was measured by DNSA method as described by Bernfeld (1955). For the estimation of total soluble sugar content in the germinating rice seeds, method of Dubois *et al.* (1956) was followed.

Statistical analysis for the data recorded from laboratory experiment and field experiment was done following the analysis of variance technique for Factorial Completely Randomised Design (CRD) as suggested by Gomez and Gomez (1984).

RESULTS AND DISCUSSION

The hydrolysis of starch into sugars is an important prerequisite for faster emergence of the seedling. Significant differences were observed among different priming durations and priming agents with respect to starch content at 7 DAS (Table 1 and Fig.1). Irrespective of priming treatments starch content was decreased from 0 to 12 hrs priming duration. Later it was increased from 24 to 48 hrs. Highest starch content was recorded at no soaking i.e., 0 hrs (45.70 mg g^{-1}) priming duration whereas lowest starch content was recorded at 12 hrs (35.38

Table 1. Effect of different seed priming agents and soaking duration on starch content (mg g^{-1}) of paddy seedlings at 7 DAS

Seed priming agent	0 hrs	6 hrs	12 hrs	24 hrs	48 hrs	Mean
No priming	45.33	45.43	45.33	45.73	45.77	45.52
Hydro priming	46.13	39.30	37.00	37.47	37.53	39.49
Nacl @ 1%	45.03	38.60	37.17	37.43	37.70	39.19
Ascorbic acid @ 40 ppm	46.30	34.73	32.10	32.60	32.70	35.69
Gibberellic acid @ 150 ppm	45.43	30.77	27.14	27.77	28.03	31.83
Neem leaf extracts @ 2 %	46.37	39.10	36.57	37.07	37.73	39.37
P & Zn (0.5&0.1%)	45.27	34.33	32.33	33.33	33.40	35.73
Mean	45.70	37.47	35.38	35.91	36.12	
Factors	C.D.	SE(d)	SE(m)			
Factor(A)	0.57	0.28	0.20			
Factor(B)	0.48	0.24	0.17			
Factor(A X B)	1.27	0.63	0.45			

Table 2. Effect of different seed priming agents and soaking duration on alpha-amylase activity (mg g^{-1}) of paddy seedlings at 7 DAS

Seed priming agent	0 hrs	6 hrs	12 hrs	24 hrs	48 hrs	Mean
No priming	1.72	1.74	1.73	1.72	1.74	1.73
Hydro priming	1.72	2.27	2.41	2.39	2.38	2.24
Nacl @ 1%	1.73	2.26	2.38	2.37	2.37	2.22
Ascorbic acid @ 40 ppm	1.73	2.55	2.72	2.70	2.68	2.48
Gibberellic acid @ 150 ppm	1.71	2.60	2.85	2.83	2.81	2.56
Neem leaf extracts @ 2 %	1.73	2.29	2.40	2.39	2.38	2.24
P & Zn (0.5&0.1%)	1.73	2.51	2.70	2.69	2.68	2.46
Mean	1.73	2.32	2.46	2.44	2.43	
Factors	C.D.	SE(d)	SE(m)			
Factor(A)	0.03	0.01	0.01			
Factor(B)	0.02	0.01	0.01			
Factor(A X B)	0.06	0.03	0.02			

mg g^{-1}) priming duration indicating faster hydrolysis of starch at 12 hrs when compared to 6, 24 and 48 hours of seed priming.

Among the priming agents, highest starch content was noticed in no priming treatment (45.52 mg g^{-1}) and the lowest starch content was recorded in the seeds primed with gibberellic acid @150 ppm (31.83 mg g^{-1}) which might be due to GA induced faster breakdown of starch. Interaction effect among the priming duration and priming agents was found to be significant. Starch content was significantly lower in the seedlings primed with GA @ 150 ppm for 12 hours (27.14 mg g^{-1}).

The above results, indicated that priming of seeds with GA @150 ppm for 12 hours resulted in lower starch content showing faster breakdown of starch into sugars. Similar findings of decrease in the starch content in the GA primed rice seedlings was previously reported by Pavani *et al.* (2018) which might be due to increased alpha amylase activity which caused rapid hydrolysis of starch into sugars.

Table 3. Effect of different seed priming agents and soaking duration on total sugars (mg g^{-1}) of paddy seedlings at 7 DAS

Seed priming agent	0 hrs	6 hrs	12 hrs	24 hrs	48 hrs	Mean
No priming	16.37	16.17	16.23	16.10	16.27	16.23
Hydro priming	16.23	20.43	22.33	21.93	21.37	20.46
Nacl @ 1%	16.27	20.10	23.00	22.13	21.40	20.58
Ascorbic acid @ 40 ppm	16.13	21.93	25.23	24.37	24.03	22.34
Gibberellic acid @ 150 ppm	16.20	25.17	28.30	27.60	27.60	24.97
Neem leaf extracts @ 2 %	16.33	20.37	22.07	21.37	21.87	20.40
P & Zn (0.5&0.1%)	16.30	22.00	25.70	24.90	23.77	22.53
Mean	16.26	20.88	23.27	22.63	22.33	
Factors	C.D.	SE(d)	SE(m)			
Factor(A)	0.39	0.20	0.14			
Factor(B)	0.33	0.17	0.12			
Factor(A X B)	0.88	0.44	0.31			

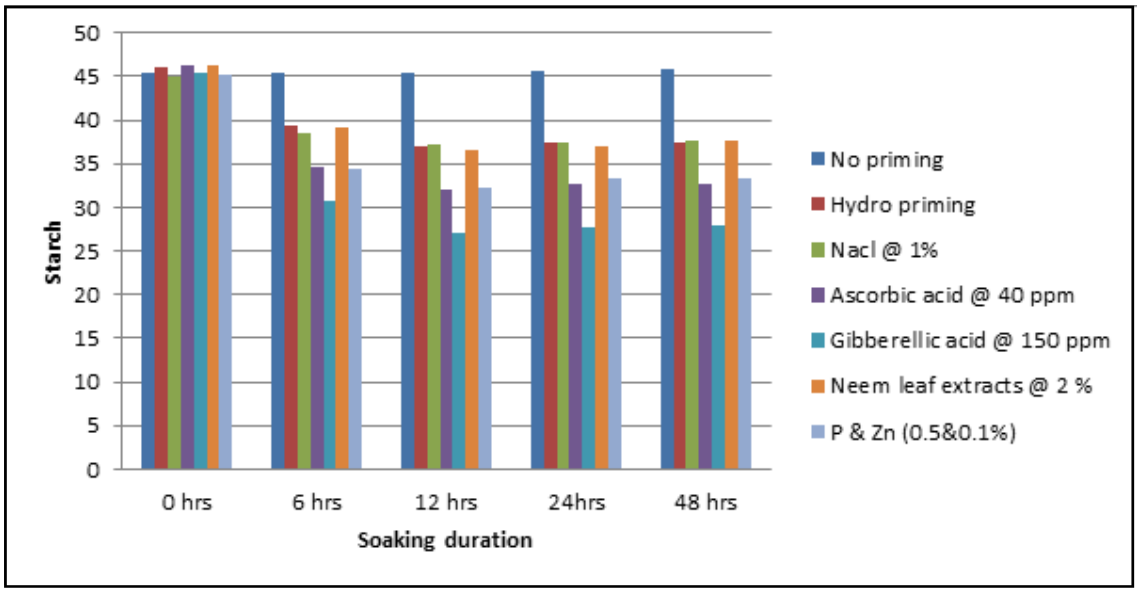

Figure 1. Effect of different seed priming agents and soaking duration on starch content (mg g^{-1}) of paddy seedlings at 7 DAS

Starch metabolism was again assessed in terms of alpha amylase activity alpha amylase activity shown significant differences with different priming durations and priming agents. At 7 DAS (Table 2 and Fig. 2). The data revealed that the alpha amylase activity obtained maximum peak in 12 (2.46 mg g^{-1}) and 24 (2.44 mg g^{-1}) hours primed seedlings whereas it was less in the seedlings with 0 (1.73) priming duration. Among the seed priming agents, lowest alpha amylase activity was observed with no priming (1.73 mg g^{-1}) treatment whereas highest alpha amylase activity was observed in seeds primed with gibberellic acid @ 150 ppm (2.56 mg g^{-1}). Seed priming with GA might have enhanced the GA synthesis in the

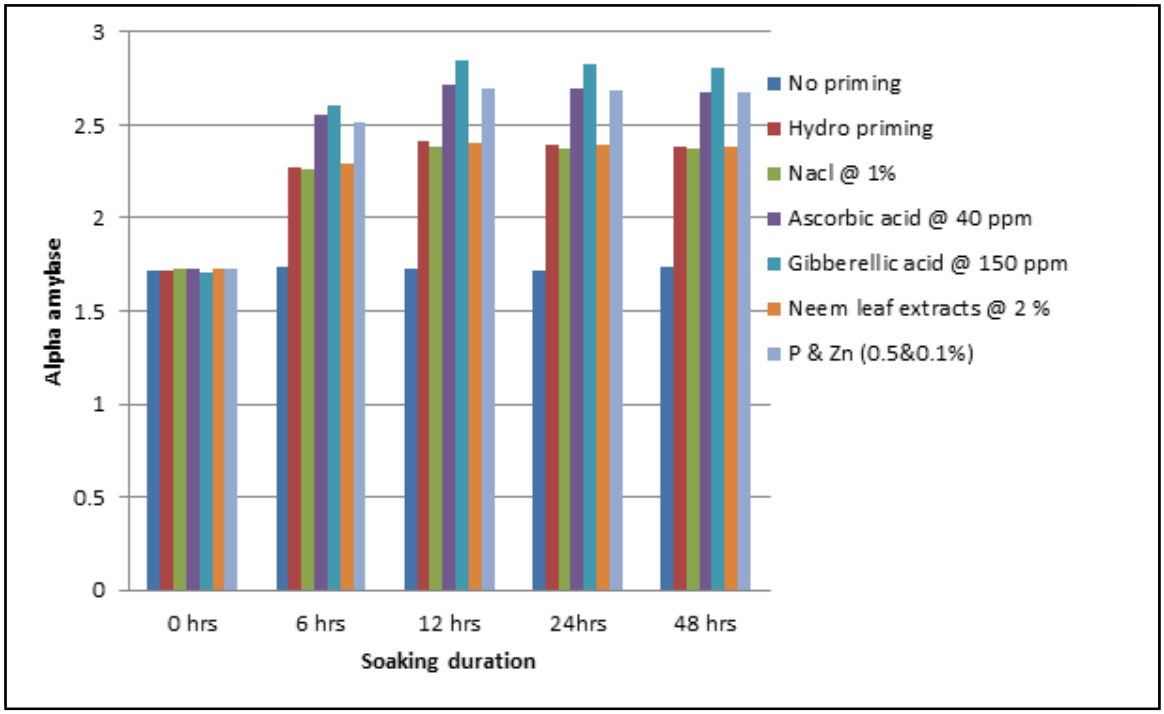

Figure 2. Effect of different seed priming agents and soaking duration on alpha-amylase activity (mg g^{-1}) of paddy seedlings at 7 DAS

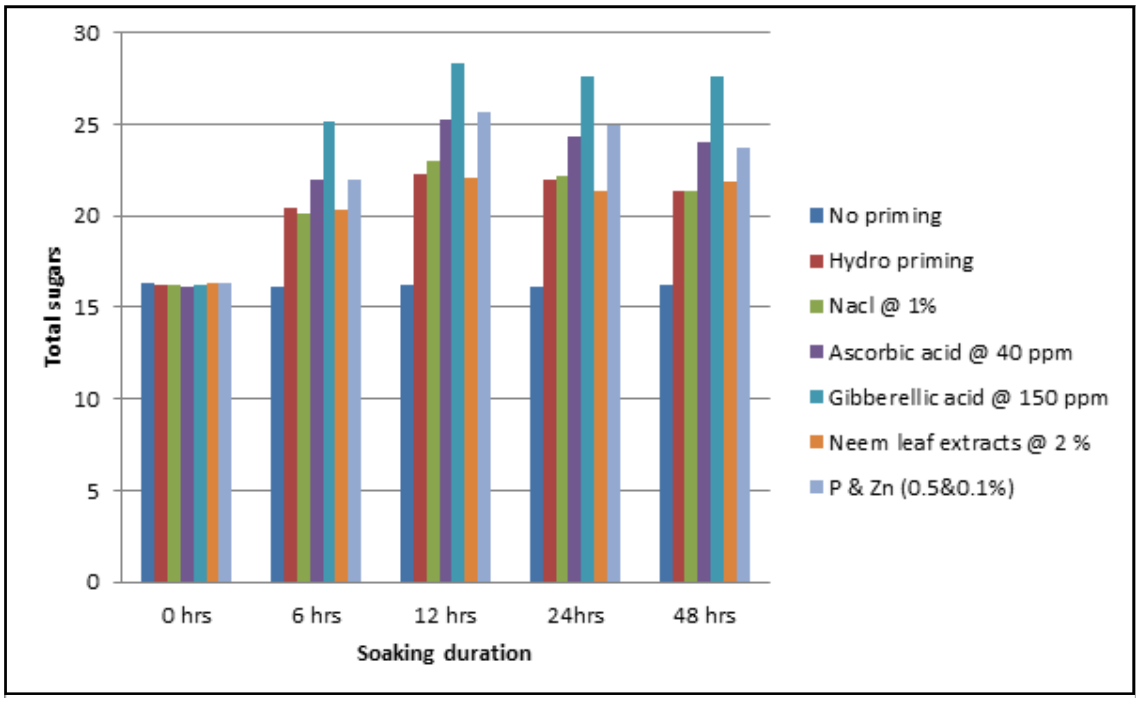

Figure 3. Effect of different seed priming agents and soaking duration on total sugars (mg g^{-1}) of paddy seedlings at 7 DAS

embryonic layer, which in turn increased alpha amylase activity. Interaction effect among the priming duration and priming agents was found to be significant. Highest alpha amylase activity was recorded in seedlings primed with GA @ 150 ppm for 12 hours (2.85 mg g^{-1}) whereas lowest in non-primed seeds.

Acharya *et al*. (1990) reported that pre-soaking of seeds with GA increased rice seedling growth and enhanced hydrolysis of starch as well as the activities of alpha amylase and beta amylase enzymes when compared to non-primed seedlings and these are in accordance with the results of the present investigation.

The data regarding total soluble sugars is presented in the Table 3 and Fig.3. Among different priming durations and priming agents with respect to total sugar content at 7 DAS shown significant differences. Total sugars were highest at 12 hours (23.27 mg g^{-1}) priming duration whereas, it was lowest in seedlings with no priming treatment (16.30 mg g^{-1}). The data pertaining to priming agents revealed that total sugar content was lowest in no primed seedlings which might be due to slower hydrolysis of starch to sugars whereas highest total sugars were recorded in the seeds primed with gibberellic acid @ 150 ppm (24.97 mg g^{-1}) which might be due to increase in the alpha amylase activity which was regulated by GA synthesized in the embryonic layer. Interaction effect among the priming duration and priming agents was found to be significant. Highest total sugar content was recorded in seedlings primed with GA @ 150ppm for 12 hours (28.30 mg g^{-1}) where as lowest was observed with non-primed seeds.

Higher levels of total sugar content in the GA primed rice seedlings was previously reported by Sukifto *et al.* (2020) which might be due to enhanced alpha amylase activity which caused rapid mobilization of food reserves and the results of the present study are in conformity with published reports of Wang *et al.* (2016).

CONCLUSION

Active degradation of starch content into sugars with production of higher levels of alpha amylase in BPT 5204 seedlings was observed at 12 hrs of soaking duration. Further, seed priming with GA @ 150ppm resulted in lower starch content, higher alpha amylase activity and higher total sugar content indicating the GA induced rapid metabolism of starch by increasing alpha amylase activity which in turn lead to higher total sugar content. The increased sugar content will accelerate the metabolic processes leading to rapid cell division and elongation. In addition to GA priming, all the priming treatments except no priming were found to influence positively the metabolic processes in the seeds leading to faster hydrolysis of starch into sugars.

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