# INFLUENCES OF BIO- SEED TREATMENT IN PEROXIDASE ACTIVITY DURING GERMINATION OF RICE HYBRID CORH 4 AND ITS PARENTAL LINES SEEDS

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**Abstract:** The rice hybrid CORH 4 and its parental lines COMS 23A (female ) and CB 174R (male) seeds were bioprimed with beneficial bio-agents *viz*., 4% *Pseudomonas fluorescens*, 20% liquid *Azospirillum*, 15% liquid phosphobacteria and 20% liquid Azophos, individually for the duration of 12 h. Unprimed and hydro-primed seeds were considered as control. After biopriming, the seed were carried over to germination and the antioxidant peroxidase enzyme activity was measured at 12h, 24h, 36h and 48h of germination. Irrespective of treatments, an increasing trend in peroxidase activity was observed with increase in germination period. After 48h of germination, seeds bioprimed with 4 per cent *P. fluorescens* for 12 h registered the maximum peroxidase content (0.435, 0.471 and 0.466 OD value) whereas unprimed seed registered minimum content (0.346, 0.365 and 0.355 OD value) in COMS 23A, CB 174R and CORH 4, respectively

**Key words:** Antioxidant, Bio-agnets, biopriming, peroxidase

**Introduction:** Peroxidase is an antioxidant scavenging enzyme. In seeds, priming appears to strengthen the defense system by means of increasing peroxidase activity during storage [4]. But in germination, increasing trend of peroxidase activity is indication of enhancement germination process. Bio priming seed treatment is controlled hydration of seed with beneficial bioagent solution. The beneficial micro organisms enhance the germination due to their plant growth promoting activity. So, the following experiment was conducted with aim of analyzing the influence of bio seed treatment in rice seed germination through peroxidase activity.

**Methodology:**

**Seed biopriming:** The rice hybrid CORH 4 and its parental lines COMS 23A (female) and CB 174R (male) seeds were soaked with beneficial bio-agents solution *viz*., 4% *Pseudomonas fluorescens*, 20% liquid *Azospirillum*, 15% liquid phosphobacteria and 20% liquid Azophos, individually for the duration of 12 h. after 12h of imbibitions the seed were remove from the solution and shade dried to original moisture content at room temperature. Unprimed and hydro-primed seeds were considered as control. After biopriming, the seed were carried over to germination and the antioxidant peroxidase enzyme activity was measured at 12h, 24h, 36h and 48h of germination.

**Assay of peroxidase (OD value):** Two replicates of 500 mg pre-germinated seed samples were homogenised in 5 ml of 0.25 M Tris buffer (pH 6.0) and centrifuged at 10000 rpm for 10 min at 5 $^0$C to extract enzymes. With 0.4 ml of enzyme extract, 0.5 ml of 1 % $H_2O_2$ and 0.5 ml of 0.5 % aqueous solution of pyrogallol were added and incubated for 10 min at 25 $^0$C. After which, the reaction was stopped by adding 0.5 ml of 5 % (v/v) $H_2SO_4$. The OD value at beginning and after 10 min was measured at 420 nm in spectrophotometer. The peroxidase activity was expressed as difference in OD/10 min [3]. "Peroxidasae activity"

$$= \frac{\text{Difference in OD value}}{\text{10 min}} \times \frac{1000}{500} \times 60$$

**Result and discussion:** The peroxidase activity was significantly influenced by biopriming treatment, germination period and its interaction. The seeds bioprimed with 4 per cent *P. fluorescens* for 12 h ($T_2$) recorded the maximum activity of 0.387, 0.406 and 0.405 OD value while the minimum activity of 0.270, 0.312 and 0.284 OD value was registered by nonprimed seeds ($T_0$) of COMS 23A, CB 174R and CORH 4, respectively.

An increasing trend in peroxidase activity was observed with increase in germination period. With increase of germination from 0 h to 48 h, peroxidase content was increased in COMS 23A (from 0.298 to 0.402 OD value), CB 174 R (from 0.319 to 0.422 OD value) and COMS 23A (from 0.308 to 0.417 OD value). Among the interaction, the maximum peroxidase content (0.435, 0.471 and 0.466 OD value) was measured in seeds bioprimed with 4 per cent *P. fluorescens* for 12 h after 48 h of germination ($T_2$× 48 h) and it was minimum (0.216, 0.255 and 0.234 OD value) in nonprimed seeds at 0 h of germination ($T_0$× 0h) of COMS 23A, CB 174R and CORH 4, respectively. When compared to nonprimed seeds and other treatments, rapid increase in peroxidase content from 0 h to 48 h was observed in the seeds bioprimed with 4 per cent *P. fluorescens* for 12 h (Table 1).Irrespective of treatments, antioxidant enzyme peroxidase (PO) (Figure. 1) activity was increased with increase of germination period. The activities of several anti-oxidative and hydrolytic enzymes raised considerably after the start of seed imbibitions [1], [2]. The increase in peroxidase might be due to the fact that food reserves were broken down and passed on to embryo. Peroxidase activity might be different according to plant species and variety, tissues and even organelles

of a cell and this enzyme complex has a close relation with germination events [5].

**Conclusion:** Generally, peroxidase enzyme was induced in response to wounding in plant. During germination, the micropylar endosperm was ruptured or wounded due to radical protrusion which leads to accumulation of peroxidase enzyme. In bio seed treatment the germination of seed was increased due to their plant growth promoting activity. The result of present experiment showed that bio- seed treatment enhanced the accumulation of peroxidase activity during germination.

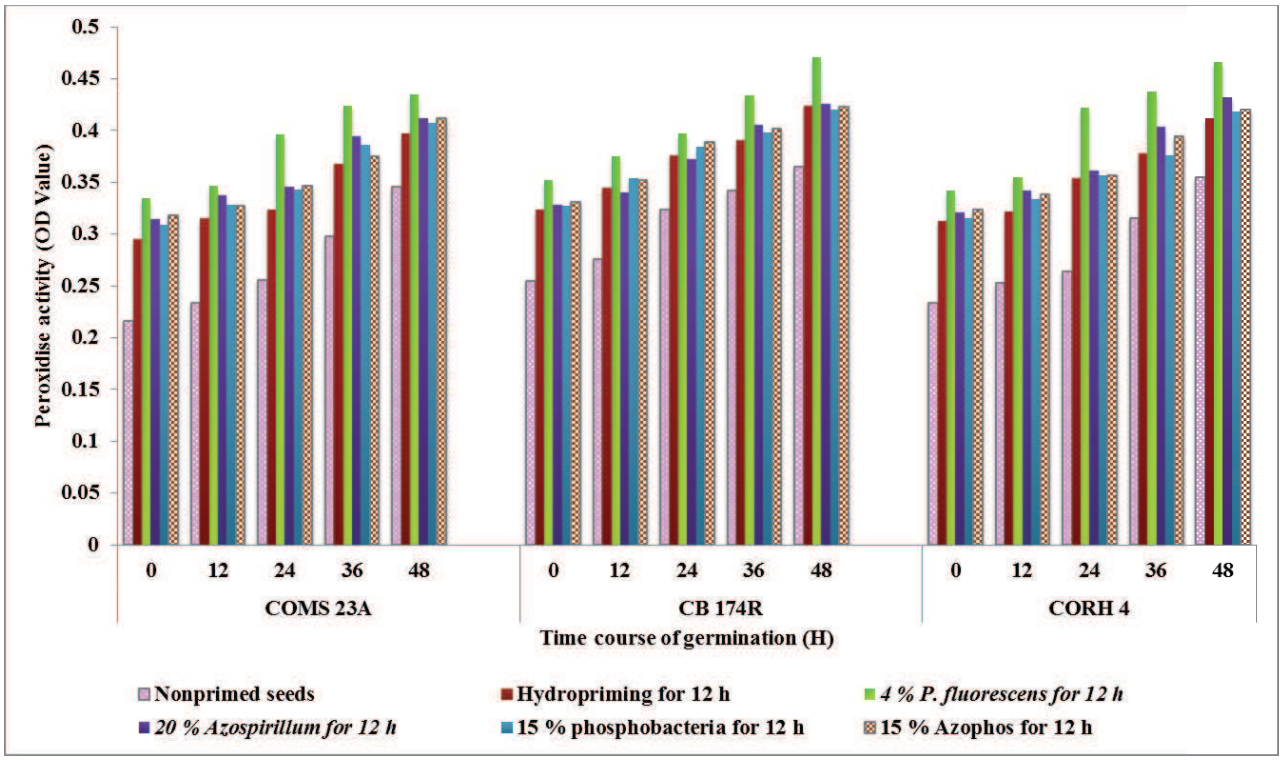


**Table 1:** Effect of seed biopriming on peroxidise activity (difference in OD at 470 nm 10 $min^{-1}$) of CORH 4 rice hybrid and its parental lines COMS 23A and CB 174R during germination.

| Biopriming treatments (T) | COMS 23A | | | | | | CB 174R | | | | | | CORH 4 | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Time course of germination (H) | | | | | | | | | | | | | | | | | |
| | 0 | 12 | 24 | 36 | 48 | Mean | 0 | 12 | 24 | 36 | 48 | Mean | 0 | 12 | 24 | 36 | 48 | Mean |
| $T_0$ | 0.216 | 0.234 | 0.256 | 0.298 | 0.346 | **0.270** | 0.255 | 0.276 | 0.324 | 0.342 | 0.365 | **0.312** | 0.234 | 0.253 | 0.264 | 0.315 | 0.355 | **0.284** |
| $T_1$ | 0.295 | 0.315 | 0.324 | 0.368 | 0.397 | **0.340** | 0.324 | 0.345 | 0.376 | 0.391 | 0.424 | **0.372** | 0.313 | 0.322 | 0.354 | 0.378 | 0.412 | **0.356** |
| $T_2$ | 0.335 | 0.347 | 0.396 | 0.424 | 0.435 | **0.387** | 0.352 | 0.375 | 0.397 | 0.434 | 0.471 | **0.406** | 0.342 | 0.355 | 0.422 | 0.438 | 0.466 | **0.404** |

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|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| $T_3$ | 0.314 | 0.337 | 0.346 | 0.394 | 0.412 | **0.361** | 0.328 | 0.340 | 0.372 | 0.405 | 0.426 | **0.374** | 0.321 | 0.342 | 0.361 | 0.404 | 0.432 | **0.372** |
| $T_4$ | 0.309 | 0.328 | 0.343 | 0.386 | 0.407 | **0.355** | 0.327 | 0.354 | 0.384 | 0.398 | 0.420 | **0.377** | 0.315 | 0.334 | 0.357 | 0.376 | 0.418 | **0.360** |
| $T_5$ | 0.318 | 0.327 | 0.347 | 0.375 | 0.412 | **0.356** | 0.331 | 0.352 | 0.389 | 0.402 | 0.423 | **0.379** | 0.324 | 0.338 | 0.357 | 0.394 | 0.420 | **0.367** |
| **Mean** | **0.298** | **0.315** | **0.335** | **0.374** | **0.402** | **0.345** | **0.319** | **0.340** | **0.374** | **0.395** | **0.422** | **0.370** | **0.308** | **0.324** | **0.353** | **0.384** | **0.417** | **0.357** |
| | **T** | | **H** | | **T x H** | | **T** | | **H** | | **T x H** | | **T** | | **H** | | **T x H** | |
| **SEd** | 0.0020 | | 0.0018 | | 0.0044 | | 0.0023 | | 0.0021 | | 0.0052 | | 0.0024 | | 0.0022 | | 0.0054 | |
| **CD (P=0.05)** | 0.0039 | | 0.0036 | | 0.0087 | | 0.0046 | | 0.0042 | | 0.0103 | | 0.0048 | | 0.0044 | | 0.0108 | |

**Treatment (T) details:**

$T_0$ –Non primed seed

$T_1$- Hydro priming for 12 h

$T_2$- Biopriming with 4 % *P. fluorescens* for 12 h

$T_3$- Biopriming with 20 % *Azospirillum* for 12 h

$T_4$- Biopriming with 15 % phosphobacteria for 12 h

$T_5$- Biopriming with 15 % Azophos for 12 h

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9. Figure 1. Peroxidise activity (difference in OD at 470 nm 10 $min^{-1}$) of bioprimed seeds of CORH 4 rice hybrid and its parental lines COMS 23A and CB 174R during germination.


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