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Proline As A Stress Indicator in Diseased Rice Plant

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Abstract

Rice is the most important food crop of the developing world. Plant diseases adversely affect the quality of plant by inducing higher level of undesirable constituents in plant. Rice Blast is a major foliar disease of rice growing area, interfere with the physiological and biochemical processes of healthy plant. The amount of proline in both young and old leaves substantially increased in plants under stressed condition. Plants were tested for proline estimations after every 30, 60, 90days of transplantation and at maturity (120 days) in both healthy and infected situations. The present investigation revealed the amount of proline in both healthy and *P. oryzae* infected rice plants. The amount of proline content was 0.52 µmole/g ,0.57 µmole/g, 0.66 µmole/g and 0.65 µmole/g in healthy plants and 0.64 µmole/g , 0.78 µmole/g, 0.82 µmole/g and 0.93 µmole/g in diseased plant at 30, 60, 90 days and at maturity(120 days) respectively.

Keywords: Proline, Metabolites, Antagonistic, Transplantation.

Introduction

Rice is the most important food crop of the developing world. To feed the increasing global population the world's annual rice production must increase to 760 x 106 tons by the year 2020. (Mohaddesi *et al* 2011)

Plant diseases adversely affect the quality of plant by inducing higher level of undesirable constituents in plant. Rice Blast is a major foliar disease of rice growing area, interfere with the physiological and biochemical processes of healthy plant. Diseases, which are generally detrimental to plant growth, adversely affect metabolism of plant and cause important modifications in plants. Such modifications may lead to accumulation or depletion of certain metabolites resulting in an imbalance in the levels of certai, metabolites.

Review of Literature

Proline

It is the odd man out, among the twenty amino acids involved in protein biosynthesis is known to accumulate in leaves of higher plants in response to a variety of abiotic and biotic stresses (Singh *et al*1973,Sahoo *et al* 2001 and Mannan *et al* 2009). The amount of proline in both young and old leaves substantially increased in plants under stressed condition. (Mostajeran *et al* 2009). It is studied in German chamomile (*Matricaria chamomilla* L.) by Amin et al (2016) and in various plants by Mukesh et al (2019) under varios abiotic stress conditions. The increase in concentration of proline in rice cultivar leaves were found to be remarkable during stress. Haudecoeur *et al* (2009) observed that in tobacco (*Nicotiana tabacum),* proline accumulation is related to susceptibility to *Agrobacterium tumefaciens, as* proline antagonizes plant defenses by interfering with the γ-aminobutyrate-mediated degradation of bacterial quorum-sensing signals that normally increase pathogen spread .

Aim of the Study

Present investigation is aimed to study the effect of blast pathogen on Proline in rice plant in response to blast disease.

Material and Methods

All the experiments were conducted at the P.G. Department lab of Govt. College, Bundi. Effect of blast disease caused by *Pyricularia oryzae,* on rice was estimated in terms of prolinel estimation. For this purpose susceptible Pusa Basmati – 1121 cultivars were selected for study. The surface sterilized seeds of above cultivars were sown and seedlings were then transplanted in pots containing sterile soil under green house conditions. At 10 days after transplantation, 60 rice plants were inoculated

with spore suspension of *P. oryzae* at the concentration of 10^6 conidia per ml. 60 plants were kept healthy (un inoculated) that served as healthy control. The Plants were tested for proline estimations after every 30, 60, 90days of transplantation and at maturity (120 days) in both healthy and infected situations.

Estmation of Proline (Bates *et al* 1973)

Healthy and blast diseased plant aerial parts were taken as sample. One gram of plant leaf was crushed in 10ml 3% sulphosaliycylic acid. It was centrifuged at two thousand rpm for ten minutes and clear supernatant was used. To the 2ml of leaf extract, 2ml of glacial acetic acid and 2 ml of freshly prepared acid ninhydrin was added. Contents were mixed well and heated in boiling water bath at 100^0C for one hour. Brick red colour was developed. After cooling, 4ml of toluene was added. Contents were stirred well .A toluene layer get separated. Its OD was taken at 520nm in spectrophotometer. All the reaction mixture except the plant sample was taken as blank. Calculation was done using standard curve prepared with D-proline.

Observation

The present investigation revealed the amount of proline in both healthy and *P. oryzae* infected rice plants. The amount of proline content was 0.52 µmole/g,, 0.57 µmole/g, 0.66 µmole/g and 0.65 µmole/g in healthy plants and 0.64 µmole/g, 0.78 µmole/g, 0.82 µmole/g and 0.93 µmole/g in diseased plant at 30, 60, 90 days and at maturity(120 days) respectively. The observation on proline content at different growth stages of rice plant is presented in table .The amount of proline in infected tissues was found to be more than healthy ones.

Considering diseased condition of plant as important biotic stress, the quantitative analysis of proline was investigated in present piece of work. The results of the investigation revealed that there was increase in the amount of proline in diseased plant over the healthy plant and this trend was followed up to the maturity of the plant. The percent increase in proline content in diseased plant over healthy plant was reported to be ranging between 18.75 %t to 30.10% with a mean value of 23.82%.

Table: Proline content in rice plant in µmole/g

S. No.	Time Interval	Proline content in Healthy Plants in µmole/g	Proline content in Diseased Plants in µmole/g	% increase over healthy plants
1	After 30 days	.52	.64	18.75%
2	After 60 days	.57	.78	26.92%
3	After 90 days	.66	.82	19.51%
4	After 120 days	.65	.93	30.10%

Result and Discussion

The result of present investigation that is the percent increase in proline content in diseased plant over healthy plant was reported to be ranging between 18.75 %t to 30.10% with a mean value of 23.82%., was in agreement with the results observed under various stressed condition of plant as given by Mohanty and Sridhar, (1982) and Radwan *et al* (2007) in viral infection. The proline content of *Colletotrichum* infected *Phaseolus vulgare* plant, raised from 2% to 4% in an observation made by Tanvernier *et al* (2007). Nicolas *et al* (2011) observed that the proline biosynthesis was altered in Arabidopsis tissue in response to *Pseudomonas syringe.*

Proline is an amino acid, which is though not involved in the synthesis of protein but reflects its importance at the time of stress. It acts as a cytoplasmic osmoticum and plays an important role in osmoregulation when the plants are subjected to stress condition (Mannan *et al* 2009). While the Proline metabolism has been widely studied in response to abiotic stresses (Hare and Cress, 1997; Verbruggen and Hermans, 2008; Szabados and Savouré, 2010), few investigations have characterized it under conditions of pathogen attack.

Conclusion

Comparative studies on proline changes during pathogenesis of infected plant as compared to healthy plats has often helped in understanding the nature and mechanism of resistance, which could be exploited in searching for disease resistant genotypes and breeding for disease resistance. This study will help to understand some aspects of biochemical defense mechanisms, operating in the host.

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Figure
Comparison of PROLINE Content µmole/g in Rice Plant after *P. oryzae* infection at Different Growth Stages