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Impact of seed priming with fruit parts extracts on biochemical parameters in blackgram seeds (*Vigna mungo* L.)

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Abstract
The present investigation was carried out to know the impact of papaya and pomegranate fruit part extract on the seed quality parameters of blackgram. The blackgram variety CO 6 was soaked in papaya peel (5%), papaya pulp (5%), papaya seed (2.5%) and pomegranate peel (7.5%), pomegranate seed (2.5%) extracts for one hour. The treated seeds along with control and hydroprimed seeds were evaluated for the enzyme activity under laboratory condition. The study revealed that the seed treated with papaya seed extract 2.5 per cent showed the higher activities of enzymes like dehydrogenase (2.79 OD value), catalase (4.19 μmol H_2O_2 min^{-1} g^{-1} protein), peroxidase (3.13 U mg^{-1}protein min^{-1}), antioxidant activity (81.66%) and superoxide dismutase (71.03 U mg^{-1} protein min^{-1}) as compared to other treatments and control seeds.

Keywords: Seed quality enhancement; blackgram; papaya; pomegranate fruit part extracts; enzyme activity

1. Introduction

Blackgram (*Vigna mungo* L.) is one of the important pulse crops in India. It is also called as urd bean and belongs to family Fabaceae (Leguminaceae). It contains the vegetable protein and supplement to cereal based diet to the population. It is the major source of protein which is about 26% which contribute to a major portion of the vegetarian diet. It contains almost three times protein that of cereals and ranks fourth among the major pulses cultivated in India. However, the area, production and productivity of blackgram are 4.48 million hectares, 2.83 million tonnes and 632 kg ha^{-1} respectively (Indiastat, 2016-17) [11].

Seed deterioration is complex and irreversible physiological processes that lead to loss of seed viability and vigour. It doesn't differ substantially from degeneration or the senescence and includes all the progressive detrimental changes that occur in seeds as they die (Bhavyasree and Vinothini, 2019) [4]. Seed invigoration techniques are used to enhance vigour and viability of seeds (Vinothini and Bhavyasree, 2019) [26, 27]. Seed priming is a controlled hydration process that involves exposing seeds to low water potentials that restrict germination (radicle protrusion), but permits pregerminative physiological and biochemical changes to occur (Heydecker and Coolbear, 1977; Bradford, 1986; Khan, 1992) [10, 6, 12]. Upon rehydration, primed seeds may exhibit a faster rate of germination, more uniform emergence, and greater tolerance to environmental stresses (Vinothini *et al.*, 2019) [26, 27].

Fruits and vegetables are a rich source of antioxidants and even the wastes or byproducts from a food processing unit such as seed and peel of some fruits contain higher amount of antioxidants (Okonogi *et al.*, 2007) [19]. Papaya (*Carica papaya* L.) it has been used as a medicinal plant as well as fruit. Papaya fruit is a good source of carotenoids, vitamin C, thiamine, riboflavin, niacin, vitamin B6 and vitamin K. So, the papaya peel is having a high amount of antioxidant potential due to the presence of phenolic compounds. High amount vitamin C (ascorbic acid), vitamin A, and some other minerals in papaya pulp (Wall, 2006) [8] as well as papaya seeds rich source for alkaloids, saponins, tannins and phenolics (Okoye, 2011) [20]. Similarly pomegranate (*Punica granatum* L.) fruit is a nutrient dense food source rich in phytochemical compounds. According to Li *et al*. (2006) [15] pomegranate peel contains high amount of tannins, flavonoids and other phenolic compounds. Pomegranate seeds also contain the nutraceutical components such as sterols, γ-tocopherol, punicic acid and hydroxyl benzoic acids (Liu *et al*., 2009) [16]. So, the antioxidants have the ability to protect and prevent oxidative deterioration of lipids and also having the ability to maintain the structural and functional integrity of cells in seeds. In seeds treated with 2.5% papaya seed extract, the enhancement of the physiological and biochemical changes in terms of field emergence, germination percentage, seedling length, dry matter production, vigour index and α- amylase activity in blackgram seeds was observed (Poovarasan *et al*., 2019) [23]. In this view to gain a better understanding of the effect of fruit-based antioxidants of papaya and pomegranate, on enzyme activity of blackgram, this study was conducted.

2. Materials and Methods

Genetically pure blackgram seeds variety of CO 6 was procured from Department of Pulses, Tamil Nadu Agricultural University, Coimbatore, which represent the source material for evaluating the seed treatment with fruit part extracts on biochemical parameters. The fruit part extracts of papaya peel (5%), papaya pulp (5%), papaya seed (2.5%) and pomegranate peel (7.5%), pomegranate seed (2.5%) were used for seed treatment. Seeds were treated with the extracts and evaluated along with water soaking and non treated seeds served as control. Seeds were soaked in the fruit part extracts for one hour in 1:1 ratio of seed to the solution (weight: volume). After treatments, the seeds were removed, rinsed with water, shade dried back under room temperature to its original moisture content and the following enzyme activity was assessed. The dehydrogenase activity was analyzed by Kittock and Law (1968) [13]. Catalase activity was estimated in the treated seeds and control seeds (Aebi, 1984) [1]. The peroxidase activity was measured by adopting the protocol of Malik and Singh, (1980) [17]. Antioxidant activity (DPPH method) was analyzed as per the method of Blois (1958) [5] and the superoxide dismutase activity of seeds was estimated in following the method of Beauchamp and Fridovich, (1971) [3]. The data was analyzed statistically adopting the procedure given by Panse and Sukhatme (1967).

2.1 Preparation of fruit parts extraction

Papaya and pomegranate fruits were obtained from the orchard of Tamil Nadu Agricultural University, Coimbatore. The fruits are washed with water and the parts were separated like papaya - peel, pulp and seed and pomegranate - peel and seed. Papaya pulp was used as such without drying because of its higher moisture content. The separated fruit parts of papaya (peel and seed) and pomegranate (peel and seed) were shade dried for 4 - 5 days followed by sun drying for 3 - 5 days after the dried parts are ground by a mini dhall mill in the Department of Food Process Engineering, Tamil Nadu Agricultural University, Coimbatore. Preparing the Fruit parts extract with different concentrations by weight per volume with distilled water.

3. Results and Discussion

Seed treated with different fruit part extract has shown highly significant differences were observed in the dehydrogenase activity. In the highest dehydrogenase activity (2.79 OD value) observed in papaya seed extract 2.5 per cent followed by pomegranate peel extract 7.5% (2.55 OD value) and papaya peel extract 5% (2.52 OD value). The lowest dehydrogenase activity (1.58 OD value) registered in non primed seeds (Fig 1). since papaya seed extract contains high antioxidant activity and hydroxyl free radical scavenging activities (Zhou *et al*., 2011) [29]. So naturally, antioxidants have the capability to protect and prevent oxidative deterioration of lipids in the seeds (Mathur, 1997) [18]. So, the antioxidants were involved in preventing, repair mechanism and also maintained the integrity of the cells in seeds. An increased dehydrogenase activity was observed in primed seeds compared to controls.

Among the treated seeds, the catalase activity showed a highly significant difference when compared to other treatments. The maximum enzyme activity (4.19 µmol H_2O_2 min^{-1} g^{-1} protein) was observed in papaya seed extract 2.5 per cent followed by pomegranate peel extract 7.5% (4.11 µmol H_2O_2 min^{-1} g^{-1} protein) and the lowest activity was observed in non primed seeds (2.45 µmol H_2O_2 min^{-1} g^{-1} protein) (Table 1). Catalases are good scavenging enzymes involved in free radical mechanism on lipid peroxidation and protect the mitochondrial components from oxidative damage (Chander and Kapoor, 1990). So, the papaya seed extract has a rich source for antioxidants in it. In this view seeds primed with the papaya seed extract can reduce the seed deterioration and also increase the catalase activity in the primed seeds.

Significant differences were observed for peroxidase activity among the treatments. Significantly highest activity (3.13 U mg^{-1}protein min^{-1}) was recorded in 2.5 per cent papaya seed extract. While minimum activity (1.05 U mg^{-1} protein min^{-1}) was observed in nonprimed seeds (Table 1). Seed primed with fruit extract can increase the enzyme activity. Peroxidase can detoxifying Reactive Oxygen species (ROS), and other functions in the cell including cell wall lignification and degradation, auxin catabolism, defence responses to insects, pathogens. The peroxidase activity was positively associated with the germination percentage in maize (Leprince *et al*., 1990) [14].

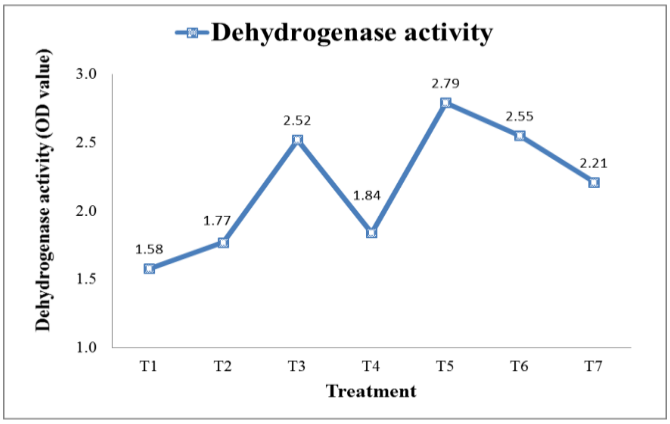

Fig 1: Influence of seed priming on dehydrogenase activity in blackgram

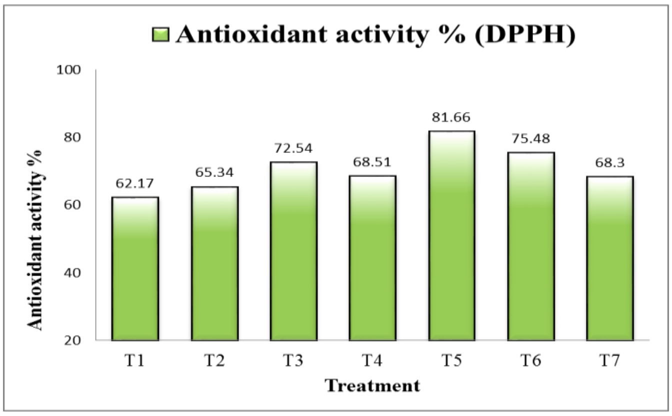

Fig 2: Influence of seed priming on antioxidant activity % in blackgram

Table 1: Influence of seed priming on antioxidant activities in blackgram

Treatment	Catalase activity (µmol H_2O_2 min^{-1} g^{-1} protein)	Peroxidase activity (U mg^{-1}protein min^{-1})	Superoxide Dismutase (U mg^{-1} protein min^{-1})
T_1	2.45	1.05	51.85
T_2	2.98	1.50	62.25
T_3	3.55	2.87	67.22
T_4	2.99	2.29	63.01
T_5	4.19	3.13	71.03
T_6	4.11	2.99	70.69
T_7	3.32	2.43	66.10
Mean	3.37	2.32	64.59
SEd	0.036	0.030	0.861
CD (P=0.05)	0.075	0.062	1.791

T_1 - Control	T_2 - Water soaking	T_3 - Papaya peel extract - 5%	T_4 - Papaya pulp extract - 5%
T_5 - Papayaseed extract - 2.5%	T_6 - Pomegranate peelextract - 7.5%	T_7 - Pomegranate seed extract - 2.5%	

Antioxidant activity differed significantly in different priming treatments. The highest antioxidant activity (81.66%) was registered in 2.5% of papaya seed extract followed by pomegranate peel 7.5% (75.48%) with an increase of 31.3% over nonprimed seed (62.17%) and 24.9% over hydropriming (65.34%) (Fig. 2). The papaya seed extract has a high amount of antioxidant potential and also for having the strongest DPPH and hydroxyl free radical scavenging activities (Zhou *et al*., 2011) [29]. Scavenging enzymes can directly involve in the detoxification of H_2O_2 and ROS. In may be a reason for the high amount of antioxidant activity in treated seeds.

Superoxide dismutase (SOD) differed significantly with treatments. The maximum activity was observed in 2.5 per cent papaya seed extract (71.03 U mg^{-1} protein min^{-1}) which were on par with pomegranate peel extract 7.5 per cent (70.69 U mg^{-1} protein min^{-1}) and nonprimed seeds recorded lowest activity (51.85 U mg^{-1} protein min^{-1}) (Table 1). Among the enzymes (SOD) involved in the quenching of the ROS and also the first defence line against the oxidative stress (Pompeu *et al.*, 2008) [22]. Similar result was obtained in priming treatments which stimulated the superoxide dismutase activity and other ROS scavenging enzyme activity in cauliflower seeds by Fujikura and Karssen (1992) [9] sunflower (Smok *et al.*, 1993; Chojnowski *et al.*, 1997; Bailly *et al.*, 2000) [24, 8, 2] and tomato (Van Pijlen *et al.*, 1995) [25].

4. Conclusion

The present study showed that papaya seed extract 2.5% recorded the maximum enzyme activity like dehydrogenase activity, catalase, peroxidase, superoxide dismutase and antioxidant activity followed by pomegranate peel extract 7.5 %. The high enzyme activity will reflect on the physiological parameters of the seed. We can conclude that 2.5 per cent of papaya seed extract can be effectively used in seed treatment for blackgram**.**

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