Journal of Oilseed Brassica, 7 (1) : 63-67, January 2016

Antioxidant potential in seed meal of different Indian mustard genotypes

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(Received: 17 Nov 2015; Revised: 23 Nov 2015; Accepted: 27 Nov 2015)

Abstract

Indian mustard [*Brassica juncea* (L.) Czern & Coss.] is a valuable source of antioxidants. Total antioxidant activity alongwith Fe^{2+} chelating activity and radical scavenging activity in methanolic extracts of seed meal was determined in ten different genotypes. The methanolic seed meal extract exhibited a concentration and genotype dependent elimination of DPPH free radical. All the genotypes showed about 50 % inhibition in 3 mg of dry seed meal. As the concentration of seed meal increased, there was corresponding increase in eradiction of free radical. The highest antioxidant activity (22.45 mg/g) and Fe^{2+} chelating activity (36.00 %) was observed in the genotype LES-51. The Fe^{2+} chelating activity varied from 20.53 % (LES-50) to 36.00 % (LES-51).

Keywords: *Antioxidant activity, DPPH free radical, Fe^{2+} chelating activity, Indian mustard*

Introduction

Indian mustard [*Brassica juncea* (L.) Czern & Coss.] is an important oilseed crop belonging to family Brassicaceae. This is in high demand for processing into animal feed and vegetable oils due to its favorable nutritional characteristic and it has much higher content of protein and phenolic compounds compared to other oilseeds (Naczk *et al.*, 1998). Mustard oil is the third-most important vegetable oil only after soybean and oil palm (Anuradha *et al.*, 2012). The presence of phenolic compounds are undesirable because they can cause bitterness, astringency and dark colour in protein products, but now these are emerging as value added products, as they exhibit antioxidant property (Das *et al.*, 2009). The seed meals of mustard also have flavonoids, tocopherols, ascorbic acid etc which exhibit antioxidant properties (Kumar *et al*., 2014). Thus, Indian mustard seed is an important source of natural antioxidants in the food industry as well as in the livestock industry for animal feeds.

Oxidative damage caused by the reactive oxygen species (ROS), such as superoxide, hydrogen peroxide and hydroxyl are physiological metabolites that commonly exist throughout the body (Bagul *et al.*, 2005). The balance between the production and neutralization of ROS by antioxidants is very delicate, and disruption in this balance leads to the over production of ROS. At this stage cells start to suffer the consequences of oxidative damage. Health problems such as heart disease, muscular degeneration, diabetes, cancer, and numerous other degenerative processes are all exacerbated by oxidative damage. It is beneficial to remove radicals to reduce the risk of diseases. Antioxidant compounds may function as free radical scavengers, complexes of pro-oxidant metals, reducing agents and quenches of singlet oxygen formation (Andlauer and Furst, 1998). They are often used in oils and fatty foods to retard their auto-oxidation. Many plant extracts and phytochemicals have been shown to have antioxidant/free radical scavenging properties. Although, food industries have used effective synthetic antioxidants, consumers of food, however, prefer natural antioxidants to synthetic antioxidants, on the basis of the assumption that natural compounds are safe (Halliwell, 2010). Therefore, in the past few years, there has been an increasing interest in determining relevant dietary sources of antioxidant compounds. The aim of this study was to estimate the total anti-oxidants, DPPH scavenging activity and metal chelating activity in the seed meal of ten different genotypes of Indian mustard.

Materials and Methods

Ten genotypes of Indian mustard were grown at Oilseeds Research Farm, Department of Genetics and Plant Breeding during the year 2014-15. At maturity freshly threshed seeds were used for study and dried at 50 °C in hot air oven. Finely powdered seeds were defatted with n-hexane (1 g/40 ml) for 6 h in Soxhlet's apparatus. The defatted seed remnant was extracted with 95 % methanol (1 g/10 ml) in a shaker at room temperature for 4 hrs,1 hr and 12 hrs for estimation of DPPH free radical scavenging activity, total antioxidant and metal chelating activity, respectively. Then the methanol extract was filtered, centrifuged at 5,000 g for 10 min and the supernatant was used for the further estimation.

Free radical 1, 1-diphenyl- 2-picryl hydrazine (DPPH) scavenging activity was monitored as described by Yen and Duh (1993). Different dilutions of the extract were incubated with 5 ml of DPPH solution (0.1 M). The samples were placed in dark for 30 min. Then the decrease in absorbance due to the scavenging of DPPH radicals by the extract was recorded at 517 nm. The percent radical scavenging capacity was calculated by the following formula: Ac-As/Ac*100 (where A_c= absorbance of control and A_s= absorbance of samples). The total antioxidant content was estimated by the method of Prieto *et al.* (1999). The methanolic extract (0.1 ml) and 1 ml reagent solution (0.6 M sulfuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate) were added and incubated at 95^0C for 90 min. The absorbance of the samples was measured at 695 nm. The antioxidant activity was expressed relative to that of ascorbic acid. The chelating activity of Fe^{2+} was estimated by the method of Hsu *et al.* (2003). The method is based on the principle of the Fe^{2+} chelating ability of the antioxidant by measuring the ferrous iron-ferrozine complex formed at 562 nm. To different concentration of extract was added 0.1 ml of 2 mM$FeCl_2.4H_2O$, 0.2 ml of 5 mM ferrozine and methanol to make up the final volume to 5 ml. The solution were mixed and allowed to react for 10 min. The absorbance at 562 nm was measured; a lower absorbance indicated a higher ferrous ion chelating capacity. Chelating activity was calculated by following equation. Scavenging effect (%) = $[1-(A_t/A_0)] * 100$ (where A_t is the absorbance of the sample and A_0 is the absorbance of the control at 562 nm).

Results and Discussion

Reactive oxygen species (ROS) generated in biological systems is dangerous to the cells by causing oxidation of its biomolecules. There is an inverse relationship between diet rich in antioxidants and the risk of cardiovascular diseases and certain forms of cancer (Kannappan and Anuradha, 2009). Plants have high antioxidant potential to scavenge or terminate ROS. In the present study seed meal of ten different Indian mustard genotypes was used for the estimation of DPPH radical scavenging activity, total antioxidative activity and Fe^{2+} chelating activity.

The DPPH free radical scavenging activity (%) in different genotypes of Indian mustard is presented in Table 1 which indicated a concentration dependent elimination of free radical in the methanolic seed meal extract. All the genotypes showed about 50 % inhibition in 3 mg concentration of dry seed meal. As the concentration of seed meal increased the eradication of free radical enhanced accordingly and

Table1. DPPH free radical scavenging activity (% inhibition) of different *B. juncea* genotypes

Conc.	% inhibition									
(mg)	Kranti	PM 29	PDZ 3	RLC 4	EJ8 118	LES 51	RL 1359	PDZ 4	EJ8 169	LES 50
1	13.9	14.6	13.6	10.1	14.1	15.0	11.3	12.1	13.9	10.5
2	37.1	39.0	36.8	34.0	39.1	30.2	33.0	35.6	37.6	32.5
3	52.0	53.8	52.0	49.2	52.7	53.8	50.6	52.3	52.5	50.0
4	68.3	69.5	67.5	57.6	65.0	69.3	61.8	68.8	68.0	60.0
5	73.5	74.9	73.2	69.1	74.0	75.0	70.7	72.0	73.7	70.0

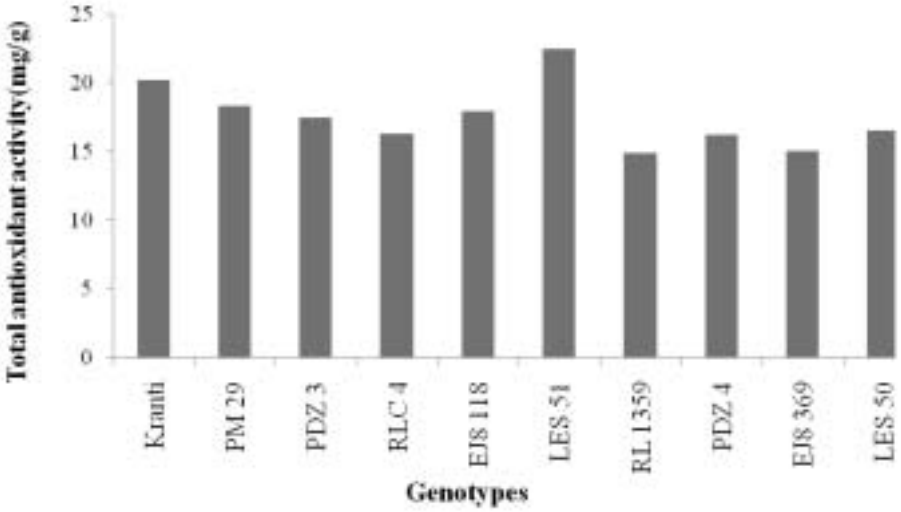

Fig 1: Total antioxidant activity of *B. juncea* genotypes.

it was concentration and genotype dependent. At 1 mg concentration of dry seed meal extract the genotypes PM-29 and LES-51 exhibited 14.64 % and 15.00 % DPPH inhibition, respectively. Similarly, at highest concentration same trend was observed in both these genotypes.

The antioxidants in defatted mustard seed extracts are effective electron\ H^+ donor and this activity contribute to the antioxidant capacity of mustard seeds. DPPH free radical scavenging activity was also reported by Manju *et al.* (2011) and Dubie *et al*. (2013) in Indian mustard. Saxena and his coworkers (2011) reported that the methanolic extract of various plant sources have better free radical scavenging activity than other solvent extracts. The IC_{50} value for free radicals scavenging activity were 2-2.5 mg of dry seed weight in Indian mustard (Dua *et al.*, 2014).

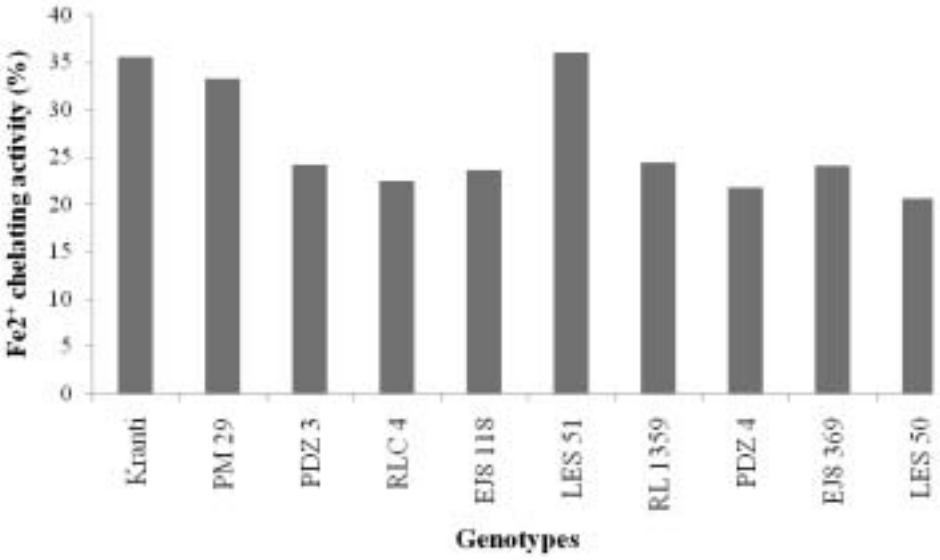

Fig 2 : Fe^{2+} chelating activity of *B. juncea* genotypes.

The total antioxidant activity ranged from 14.84 to 22.45 mg/g (Fig. 1). The highest antioxidant activity was observed in the genotype LES-51 (22.45 mg/g) followed by Kranti (20.13 mg/g) and PM 29 (18.25 mg/g). Plants have ascorbate, riboflavin, tocopherol and polyphenols with redox potential high enough to scavenge or terminate ROS (Scafer *et al*., 2003). High antioxidant activity has also been reported earlier in Indian mustard (Manju *et al*., 2011; Dua *et al*., 2014; Kumar *et al*., 2014; Kumar 2014).

Per cent chelating effect shown by the methanolic extracts of Indian mustard demonstrates that they not only have the ability to combate oxidation reactions directly but can also decelerate the oxidation reaction by chelating pro-oxidant metals. Transition metals like copper, iron and nickel are pro-oxidant and thus promote oxidation by acting as catalyst and therefore sequestering of these metal ions by antioxidants decrease their pro-oxidant potential by stabilizing their oxidized form (Fe^{3+}). The Fe^{2+} chelating activity of different genotypes varied from 20.53 to 36.00 % (Fig. 2). The highest chealting activity was recorded in the genotype LES-51 (36.00 %) followed by Kranti (35.54%), PM 29 (33.20%), RL 1359 (24.33 %) and PDZ-3 (24.14 %). The higher the antioxidant potential, the higher is the conversion of Fe^{3+} in ferric chloride to Fe^{2+}. Similar results have also been reported by Ishtiaque *et al.* (2013) in Indian mustard.

Conclusion

The results of the present study indicated that the oil free methanolic extract of Indian mustard seeds have high antioxidants potential. The study also suggested that the natural antioxidants may preferably be used for food preservation and animal feed. The Indian mustard has highest antioxidant potential in terms of antioxidative activity, DPPH free radical scavenging activity and Fe^{2+} chelating activity. This study revealed that LES-51, Kranti and PM-29 have high antioxidant potential. Due to presence of natural antioxidant compounds in Indian mustard, it has good potential for future use in food and nutraceutical supplement formulations.

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