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PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Pakistan Journal of Biological Sciences 9 (9): 1812-1816, 2006
ISSN 1028-8880

Tocopherol and Sterol Content of Some Rapeseed/Mustard Cultivars Developed in Bangladesh

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Abstract: Seed samples of 20 indigenous and developed mustard/rapeseed cultivars (*Brassica campestris, B. juncea* and *B. napus*) were analyzed for tocopherol- and sterol-content. Gas liquid chromatography and high performance liquid chromatography were used, respectively, for sterol and tocopherol analysis. Four types of tocopherols were found in mustard oil in various amount: α-tocopherol, β-tocopherol, χ-tocopherol and δ-tocopherol, ranged from 14.96 to 32.48, trace amount to 1.19, 26.12 to 66.51 and 2.02 to 5.38 μg g^{-1} lipids, respectively. Only χ-tocotrienol, among the tocotrienlos, was identified and its amount varied from 2.02 to 7.49 μg g^{-1} lipids among the cultivars. Major sterols identified and their lipid concentrations in μg g^{-1} lipid were as follows: sitosterol, 3,183 to 4,463; brassicasterol, 582 to 1,368; campesterol, 1,306 to 3,471; Δ5-avenasterol, 138 to 1,135 and cycloartenol, 119 to 1,170. This study shows that both tocopherol and sterol content differ significantly among the cultivars.

Key words: Lipid, tocopherol, sterol, rapeseed, mustard

INTRODUCTION

Mustard and rapeseed together rank the second most important vegetable oils in the world. They have been playing important role as a source of oils in human diet for centuries. They provide the most concentrated source of energy and also help in absorption of fat-soluble vitamins. Vegetables oil consumption has been increasing all over the world in recent years.

In Bangladesh, rapeseed and mustard (*Brassica* sp.) are the principal oilseed crops. They serve many other dietary purposes in Bangladeshis daily life including cooking and salad preparation. However, the seed yield of the indigenous cultivar is very low (740 kg ha^{-1}) and meets a minimum requirement of less than 15 g per capita per day compared with the optimum requirement of 35 g per capita per day (BBS, 2003). Recently, Bangladeshi plant breeders have developed several high yielding cultivars of rapeseed and mustard to satisfy the need of our increasing population. Some developed cultivars yield as much as 1500 kg ha^{-1} (Das and Malek, 2004). However, the data about the quality aspects of these high-yielding rapeseed and mustard cultivars are lacking.

The quality of edible oils can be considered from a consumer acceptance and a food technological point of view. These characteristics can be physical, such as colour, viscosity, or crystal structure, as well as chemical, such as hydrolysis, oxidation, flavour, or polymorphism (Smouse, 1994). For studying oil quality and stability, primary analysis performed on oil is determination of the fatty acid composition. This gives a preliminary indication of the quality of oil. The basis for functional and nutritional characteristics of vegetable oils is not fully understood; however, other than fatty acid composition and their position in the glycerol backbone, the levels of other minor constituents, collectively known as the unsaponifiable fractions, are some of the main factors that affect these properties (Neff *et al*., 1992, 1994). Sterols are the major constituents of the unsaponifiable fractions from most vegetable oil, whose composition in oils and fats is useful in the characterization of the oil identity (Johansson and Croon, 1981) and detection of adulteration (El-Hinnawy *et al*., 1983; Sil *et al*., 1990). Another group of unsaponifiable, known as tochopherols act as good antioxidant and thus increase the oxidative stability of oil (Wagner *et al*., 2004).

Oxidative stability of oils is very important for consumers. When oils rich in the nutritionally valuable linoleic and linolenic acids are stored, they easily react with oxygen and form hydroperoxides that decompose and give rise to ill-tasting aldehydes and ketones. This oxidation process is inhibited or delayed by antioxidants of which tocopherols is one important group (Wagner *et al*., 2004). It has, however, been demonstrated that not only tocopherols but also certain sterols, viz. those with an ethylidene side chain, act as antioxidant at high temperature. Phytosterols are also known to inhibit absorption of dietary cholesterol (Eskin *et al*., 1996; Gordon and Magos, 1983).

In previous study, I analyzed the fatty acid composition, in particular emphasis on erucic acid level and its association with other major fatty acids, in oil of these seed samples (Mortuza *et al*., 2006). I have identified some developed cultivars that contain significantly less amount of erucic acid compared with indigenous one. This finding has made me interesting to investigate in more detail some minor constituents of these oils. Keeping this importance in mind, present study was carried out to characterize different phytosterols and tocopherols of these seed oils. The aim of this study was to generate comprehensive information on these parameters for fat-quality evaluation to select desirable genotypes of locally adaptive rapeseed/mustard cultivars.

MATERIALS AND METHODS

Seed samples of 20 mustard cultivars were collected from Bangladesh Agricultural Research Institute (BARI), Joydevpur and Bangladesh Institute of Nuclear Agricultural (BINA), Mymensingh, Bangladesh (Table 1). Seeds were cleaned manually, sundried, packed in paper bags and kept at room temperature.

Extraction of oil: Oil was extracted in duplicates according to Hara and Radin (1978) with slightly modified by Savage *et al.* (1997). Briefly, 10 g mustard seed was put into a steel tube containing three steel balls to facilitate proper homogenization. Then 30 mL of HIP (Haxane: Isopropanol, 3:2 v/v) solution was added. The tube was placed in ashaker for homogenization for 1 h at room temperature. The homogenate was filtered to a Buchner funnel, which was connected to mild vacuum. The tube was washed twice with 20 mL HIP solution each time. Next the filtrate was transferred to a separatory funnel and vacuum flask was washed with 20 mL HIP solution, which was added to separatory funnel. About 45 mL 6.7% Na_2SO_4 solution was added to the separatory funnel and the content was shaken vigorously for proper mixing. The funnel was kept undisturbed to allow the mixture to settle into two phases. The upper hexane layer was collected by thinpipet and dried with anhydrous Na_2SO_4 and collected to an evaporatory flask. The content of the flask was then rotary-evaporated under reduced pressure at 30°C. Pure oils were stored at -20°C for subsequent analysis.

Saponification for sterol analysis: Saponification was made by mixing about 10 mg of extracted oil and 20 μg of 5-α-cholestane (Sigma Chemical Co., St. Louis, USA) as internal standard with 1 mL 2 M KOH in absolute ethanol in a glass tube with a stopper and clip. The tubes were kept at 60°C for 45 min in a water bath under shaking. The reaction was stopped by cooling the tubes under running cold water and 1 mL water, 2 mL hexane and 0.1 mL ethanol were added. The tubes were shaken vigorously and centrifuged at 3000 rpm for 3 min. The upper hexane layer was transferred to a small glass tube with a ground glass stopper. The solvent was evaporated under stream of nitrogen and derivatised to trimethylsilyl (TMS) ethers for subsequent analysis by GC according to Savage *et al.* (1997).

Table 1: Cultivars of oil yielding *Brassica* sp. (mustard/rapeseed) with their origin

Cultivars/Advanced mutant lines	Species	Origin of the cultivars
Cv. Tori-7	*B. campestris* L. var. *Toria*	Developed from the indigenous collection of germplasm by BARI.
Cv. Binasarisha-3	*B. napus* L.	A gamma-ray induced mutant variety from Nap-3, an exotic line, developed by BINA.
Cv. Binasarisha-4	*B. napus* L.	A gamma-ray induced mutant variety from Nap-3, an exotic line, developed by BINA.
Cv. Binasarisha-5	*B. napus* L.	A gamma-ray induced mutant variety from Nap-3, an exotic line, developed by BINA.
Cv. Binasarisha-6	*B. campestris* L. var. *Yellow sarson*	A gamma-ray induced mutant variety developed by BINA.
MM22-12-98	*B. napus* L.	A gamma-ray induced advanced generation mutant line of BINA from Nap-3, an exotic line.
MM2-16-98	*B. napus* L.	A gamma-ray induced advanced generation mutant line of BINA from Nap-3, an exotic line.
MM38-6-98	*B. napus* L.	A gamma-ray induced advanced generation mutant line of BINA from Nap-3, an exotic line.
MM49-3-98	*B. napus* L.	A gamma-ray induced advanced generation mutant line of BINA from Nap-3, an exotic line.
MM34-7-98	*B. napus* L.	A gamma-ray induced advanced generation mutant line of BINA from Nap-3, an exotic line.
MM001/98	*B. juncea* L.	A gamma-ray induced advanced generation mutant line of BINA from exotic collection "Jata".
Cv. Jata	*B. juncea* L.	An Indian variety.
Cv. Agrani	*B. campestris* L. var. *Yellow sarson*	A gamma-ray induced mutant variety from the local collection, YS-52, developed by BINA.
Cv. Safal	*B. campestris* L. var. *Yellow sarson*	A gamma-ray induced mutant variety from the local collection, YS-52, developed by BINA.
Cv. Rai-5	*B. juncea* L.	Developed from the indigenous collection of germplasm by BARI.
Cv. Barisarisha-6	*B. campestris* L. var. *Yellow sarson*	Developed from the indigenous collection of germplasm by BARI.
Cv. Barisarisha-8	*B. napus* L.	Developed through introgre-ssion of different species of Brassica by BARI.
Cv. Barisarisha-9	*B. campestris* L.	Developed through selection from an Indian germplasm line by BARI.
Cv. Barisarisha-10	*B. juncea* L.	Developed through selection from local and exotic germplasm collection by BARI.
Cv. Barisarisha-11	*B. juncea* L.	Developed from the exotic collection of germplasm by BARI.

BARI = Bangladesh Agricultural Research Institute, Joydebpur, Bangladesh, BINA = Bangladesh Institute of Nuclear Agriculture, Mymensingh, Bangladesh

Preparation of TMS ether derivatives of sterols: Tri-Sil reagent (100 mL) (Pierce Chemical Co., Rockford, USA) was added to the saponified samples above and the tubes were incubated at 60°C for 45 min. Next the solvent was evaporated under stream of nitrogen and the TMS ether derivatives were dissolved in 1 mL hexane. Then the tubes were sonicated in an ultrasonic bath for 1 min and centrifuged for 3 min. Thereafter the hexane layer was transferred to another tube, avoiding any solid particles, evaporated to dryness and dissolved in 500 μL hexane and kept at -20°C until further analysis by GC.

Analysis of sterol by GC: For this purpose, a fused silica capillary column CP-Sil-5 CB (Chrompack, Middleburg, The Netherlands), length 30 m, i.d. 0.25 mm, film thickness 0.50 μm, was used. The column was connected to a Varian 3700 gas chromatograph equipped with a flame ionization detector and split injector. Helium was used as a carrier gas at an inlet pressure of 17 psi and as a make-up gas at a rate of 30 mL min^{-1}. The GC oven was run with a temperature programmed at 270°C for 10 min, rising to 300°C at a rate of 1°C min^{-1}. The detector temperature was 300°C. An HP 3396A integrator (Hewlett-Packard, Avondale, PA, USA) was used for peak integration. The sterols were identified by comparing the relative retention times of 5-α-cholestane with those of standard cholesterol, campasterol, stigmasterol, sitosterol and Δ-5-avenasterol. No response factors were used. All analyses were made duplicate.

Analysis of tocopherol by HPLC: Tocopherol content was determined by dissolving 0.1 g in duplicate extracted oil in 1.0 mL n-Heptane and injecting 20 μL into a HPLC column. Briefly, a HPLC column LiChroCART 250-4, packed with Lichrospher 100 NH_2, particle size 5 μm was coupled to a guard column LiChroCART 4-4 (Merk, KGaA, Darmstadt, Germany). The column was fitted with a Rheodyne 7125 injector and an intelligent pump model L-6200-A and a Merck-Hitachi fluorescence spectrophotometer model F-1050 (Merck, Germany; Hitachi, Japan) was used for detection of tocopherols at wavelengths of 295 and 320 nm for excitation and emission, respectively. Isocratic elution was carried out with a mixture of hexane: tertbutylmethylether: tetrahydrofuran: methanol (79:20:0.98:0.02, by vol.) at a flow rate of 1.25 mL min^{-1}. Tocopherols were quantified by using external standards with reference samples of tocopherols (Merck, Germany). A Hewlett-Packard HP 3390A integrator (Hewlett-Packard, Avondale, PA, USA) was used for calculating the peak area.

Statistical analysis: One-way ANOVA calculation (Turkey t-test) was applied to check the significant differences of parameters among the cultivars.

RESULTS AND DISCUSSION

Tocopherol content: Four types of tocopherols were found in oil in various amounts: α-tocopherol, β-tocopherol, χ-tocopherol and δ-tocopherol, ranged from 14.96 to 32.48, trace amount to 1.19, 26.12 to 66.51 and 2.02 to 5.38 μg g^{-1} lipids, respectively (Table 2). MM22-12-98 contained significantly ($p \leq 0.001$) highest amount while Barisarisha-10 contained significantly ($p \leq 0.001$) lowest amount of α-tocopherol; all other cultivars contained more or less same amount of α-tocopherol. Binasarisha-6, MM49-3-98 and MM34-7-98 were found to contain significantly ($p \leq 0.001$) highest

Table 2: The tocopherol content* (μg g^{-1} lipids) of oil extracted from rapeseed/mustard cultivars developed in Bangladesh

Cultivar	α-tocopherol	β-tocopherol	χ-tocopherol	δ-tocopherol	χ-tocotrienol	Total
Tori-7	25.44^{b}	trace	38.82^{b}	3.27^{b}	3.16^{b}	70.69^{b}
Binasarisha-3	22.01^{b}	0.25^{b}	30.92^{b}	2.24^{c}	5.18^{a}	60.61^{c}
Binasarisha-4	28.04^{b}	1.19^{a}	34.97^{b}	2.02^{c}	6.47^{a}	72.71^{b}
Binasarisha-5	26.34^{b}	trace	34.64^{b}	2.89^{c}	7.24^{a}	71.12^{b}
Binasarisha-6	26.96^{b}	0.48^{b}	32.07^{b}	5.08^{a}	4.38^{a}	68.99^{b}
MM22-12-98	32.48^{a}	trace	42.52^{b}	3.56^{b}	5.34^{a}	83.90^{a}
MM2-16-98	24.70^{b}	0.36^{b}	31.58^{b}	4.20^{b}	5.67^{a}	66.51^{b}
MM38-6-98	24.94^{b}	trace	31.18^{b}	4.02^{b}	7.34^{a}	67.48^{b}
MM49-3-98	24.14^{b}	trace	35.87^{b}	5.38^{a}	6.16^{a}	71.55^{b}
MM34-7-98	23.02^{b}	trace	29.20^{b}	4.90^{a}	7.08^{a}	64.20^{b}
MM001/98	18.15^{b}	trace	59.52^{a}	2.84^{b}	3.08^{b}	83.59^{a}
Jata	17.65^{b}	trace	45.79^{b}	2.43^{c}	2.02^{b}	67.92^{b}
Agrani	25.29^{b}	0.62^{a}	33.59^{b}	4.22^{b}	3.19^{b}	66.92^{b}
Safal	20.72^{b}	0.64^{a}	26.30^{c}	4.29^{b}	3.66^{b}	55.64^{c}
Rai-5	21.84^{b}	trace	50.05^{a}	2.88^{b}	2.30^{b}	77.08^{b}
Barisarisha-6	24.77^{b}	0.39^{b}	26.12^{c}	2.39^{c}	2.57^{b}	56.24^{c}
Barisarisha-8	25.35^{b}	trace	41.12^{b}	3.43^{b}	7.49^{a}	77.39^{b}
Barisarisha-9	23.37^{b}	trace	40.41^{b}	2.24^{c}	5.18^{a}	71.20^{b}
Barisarisha-10	14.96^{c}	trace	66.51^{a}	3.83^{b}	2.41^{b}	87.71^{a}
Barisarisha-11	19.05^{b}	trace	57.95^{a}	2.91^{b}	2.40^{b}	82.31^{a}

Note: Trace is less than 0.25, * Mean of duplicate values, ^{a,b,c} significantly ($p \leq 0.001$) different

amount of δ-tocopherol among the cultivars. On the other hand, Binasarisha-3, Binasarisha-4, Binasarisha-5, Barisarisha-6 and Barisarisha-9 contained the lowest amount of δ-tocopherol; rest of the cultivars contained moderate amount of δ-tocopherol. Surprisingly high values were obtained in χ-tocopherol content of MM001/98, Rai-5, Barisarisha-10 and Barisarisha-11 cultivars. However, apart from tocopherols, only one other compound was found in moderate amount in mustard oil. This compound was identified as χ-tocotrienol by comparing HPLC peaks with standard samples. The peak was quantified by tocopherol standard series; ranged from 2.02 to 7.49 μg g^{-1} lipids. Binasarisha-3, Binasarisha-4, Binasarisha-5, Binasarisha-6, MM22-12-98, MM2-16-98, MM38-6-98, MM49-3-98, MM34-7-98, Barisarisha-8 and Barisarisha-9 contained significantly ($p \leq 0.001$) highest amount and Jata contained the lowest amount of χ-tocotrienol among the cultivars. Total tocopherol (tocopherol plus tocotrienol) content among the cultivars varied widely; MM22-12-98, MM001/98, Barisarisha-10 and Barisarisha-11 contained significantly ($p \leq 0.001$) highest amount (82.31-87.71 μg g^{-1} lipid); Binasarisha-3, Safal and Barisarisha-6 contained significantly lowest amount (55.64-60.61 μg g^{-1} lipid) and rest cultivars contained moderate amount (64.20-77.39 μg g^{-1} lipid).

Sterol content: The major sterols identified were sitosterol, Brassicasterol, campesterol, △5-avenasterol and cycloartenol and their amount in μg g^{-1} lipids ranged from 3,183 to 4,463; 582 to 1,368; 1,306 to 3,471; 138 to 1,135; and 119 to 1,170 (Table 3). Cholesterol (data not shown in table) and stigmasterol were found in trace amount with no significant difference among the cultivars. Variation in the total amount of sterol content was also observed; however, unexpectedly low value was obtained in Barisarisha-6 (5,768 μg g^{-1}). Tori-7, MM001/98, Rai-5 and Barisarsha-10 contained significantly ($p \leq 0.001$) highest amount of sitosterol among all the cultivars; Barisarisha-6, however, contained again unexpectedly

Table 3: Quantitative composition of phytosterols *'of oil extracted from rapeseed/mustard cultivars developed in Bangladesh

Cultivar	Brassicasterol	Campesterol	Stigmasterol	Sitosterol	Δ5-Avenasterol	Cycloartenol	Total
Tori-7	1.232[a]	2.361[b]	93	4.463[a]	267[b]	161[b]	8.577[a]
	(14.36)	(27.53)	(1.08)	(52.03)	(3.11)	(1.88)	
Binasarisha-3	995[a]	2.731[b]	95	3.569[b]	768[a]	1.170[a]	9.328[a]
	(10.67)	(29.28)	(1.02)	(38.26)	(8.23)	(12.94)	
Binasarisha-4	725[a]	2.039[b]	58	3.354[b]	588[b]	119[b]	6.883[b]
	(10.53)	(29.62)	(0.84)	(48.73)	(8.54)	(1.73)	
Binasarisha-5	745[a]	2.071[b]	150	3.902[b]	263[b]	232[b]	7.363[a]
	(10.12)	(28.13)	(2.04)	(52.99)	(3.57)	(3.15)	
Binasarisha-6	1.009[a]	1.472[c]	160	3.933[b]	193[b]	271[b]	7.038[a]
	(14.34)	(20.92)	(2.27)	(55.88)	(2.74)	(3.85)	
MM22-12-98	868[a]	1.917[b]	148	3.637[a]	319[b]	158[b]	7.047[a]
	(12.32)	(27.20)	(2.10)	(51.57)	(4.53)	(2.24)	
MM2-16-98	854[a]	2.456[b]	106	3.416[b]	710[a]	148[b]	7.690[a]
	(11.11)	(31.94)	(1.38)	(44.42)	(9.23)	(1.92)	
MM38-6-98	925[a]	1.779[b]	87	3.634[b]	970[a]	197[b]	7.592[a]
	(12.18)	(23.43)	(1.15)	(47.87)	(12.78)	(2.59)	
MM49-3-98	1.029[a]	2.949[b]	110	3.920[b]	817[a]	200[b]	9.025[a]
	(11.40)	(32.68)	(1.22)	(43.43)	(9.05)	(2.22)	
MM34-7-98	903[a]	2.937[b]	115	3.587[b]	1.135[a]	218[b]	8.895[a]
	(10.15)	(33.02)	(1.29)	(40.43)	(12.76)	(2.45)	
MM001/98	1.232[a]	3.158[b]	100	4.031[a]	166[b]	125[b]	8.812[a]
	(13.98)	(35.84)	(1.13)	(45.74)	(1.88)	(1.42)	
Jata	991[a]	3.063[b]	100	3.699[b]	477[b]	143[b]	8.473[a]
	(11.69)	(36.15)	(1.18)	(43.66)	(5.63)	(1.68)	
Agrani	1.020[a]	1.306[c]	146	3.504[b]	138[b]	307[b]	6.421[b]
	(15.89)	(20.34)	(2.27)	(54.57)	(2.15)	(4.78)	
Safal	1.020[a]	1.410[c]	87	3.282[b]	168[b]	317[b]	6.284[b]
	(16.23)	(22.44)	(1.38)	(52.23)	(2.67)	(5.09)	
Rai-5	1.368[a]	3.471[a]	83	4.344[a]	176[b]	149[b]	9.591[a]
	(14.26)	(36.19)	(0.86)	(45.29)	(1.84)	(1.55)	
Barisarisha-6	582[b]	1.444[c]	99	3.183[b]	135[b]	325[b]	5.768[b]
	(10.09)	(25.03)	(1.72)	(55.18)	(2.34)	(5.63)	
Barisarisha-8	781[a]	2.318[b]	72	3.309[b]	375[b]	202[b]	7.057[a]
	(11.07)	(32.85)	(1.02)	(46.89)	(5.31)	(2.86)	
Barisarisha-9	1.141[a]	2.115[b]	133	3.623[b]	238[b]	208[b]	7.458[a]
	(15.30)	(28.36)	(1.78)	(48.58)	(3.19)	(2.79)	
Barisarisha-10	1.144[a]	2.997[b]	121	4.095[a]	201[b]	177[b]	8.735[a]
	(13.10)	(34.31)	(1.39)	(46.88)	(2.30)	(2.03)	
Barisarisha-11	1.292[a]	3.007[b]	121	3.921[b]	355[b]	193[b]	8.889[a]
	(14.53)	(33.83)	(1.36)	(44.11)	(3.99)	(2.17)	

* Mean of duplicate values. ' μg g^{-1} (%). [a,b,c] significantly ($p \leq 0.001$) different

the lowest amount. Cycloartenol content of Binasarisha-3 was found surprisingly highest among the cultivars. △5-avenasterol content of Binasarisha-3, MM2-16-98, MM38-6-98, MM49-3-98 and MM34-7-98 were significantly higher than that of the rest cultivars. Brassicasterol and campesterol content were found more or less similar among the cultivars studied with an exception that Barisarisha-6 contained the lowest amount of both brassicasterol and campesterol whereas Binasarisha-6, Agrani and Safal contained the lowest amount of campesterol only. These results are in partial agreement with earlier report (Sil *et al*., 1990).

Tocopherol-and sterol-composition as well as their individual amount in the unsaponifiable fractions are the main parameters for characterizing an oil sample. Sterol composition is mostly useful in identifying oils as well as detecting their adulteration. Besides, some sterols are also reported to act as antioxidant. Tochopherols, mainly δ-tocopherol, but also χ-tocopherol even less pronounced, are reported to act as good antioxidant and thus prolong the shelf life of oil. In this study a wide variation in amount of both sterols and tochopherols was observed among the Mustard/rapeseed cultivars. Some mustard/rapeseed cultivars, namely, Binasarisha-4, Binasarisha-5, MM 22-12-98, MM 2-16-98, MM 49-3-98 and Barisarisha-8 of *B. napus* showed considerably high oxidative stability (Mortuza, unpublished). Future studies in this area should focus on identifying the factors responsible for this high stability of oils of these developed cultivars, which has not been ascertained in this study. These mentioned cultivars and advanced mutant lines may be exploited in breeding program for further development of nutritionally better-quality locally adaptive cultivars.

ACKNOWLEDGMENTS

I acknowledge with thanks The Swedish Institute, Stockholm, Sweden, for offering me scholarship to carry out this study at the Swedish University of Agricultural Sciences, Uppsala, Sweden. My special thanks to Dr PC Dutta, Swedish University of Agricultural Sciences, Uppsala, Sweden and Dr. M.L. Das, Bangladesh Institute of Nuclear Agriculture, Mymensingh-2200, Bangladesh for providing lab facilities and seed samples, respectively.

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