Pak. J. Sci. Ind. Res. 2003 ***46****(6) 447 - 451*

LEAF PHENOLICS OF DIFFERENT VARIETIES OF TROPICAL RAPESEED AT VARIOUS GROWING STAGES

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(Received October 5, 2002; accepted May 19, 2003)

Three species of rapeseed viz RM-9-7 (*Brassica napus*), BM-1 (*Brassica juncea*) and peela raya (*Brassica carinata*) were grown using normal agronomic practices. The leaves of three species were harvested after 20,40,60,80,100 and 120 days of sowing for analysis of different polyphenols after extraction in water and methanol by spectrophotometric methods. The results revealed that maximum concentration of sinapine, total phenols, leucoanthocyanidine and procyanidine were highest after 80 days of sowing in all species except the leucoanthocyanidine content of BM-I and peela raya species where maximum concentration was recorded after 100 days of sowing. Concentrations of methanol extractable phenolics were higher than water extractable phenolics in all species. Maximum values for methanol soluble sinapine (0.243%), total phenols (0.203%), leucoanthocyanidine (0.812 Δ A 550/g) and procyanidine (Δ A 550/g) were found in RM-9-7, BM-1 and peela raya, respectively. It may be concluded that for optimum phenolics concentration in the extract, the leaves of these species should be harvested after 80 days.

Key words: Methanol extractable phenolics, Rapeseed leaves, Growing stages, Sinapine.

Introduction

Rapeseed is among world's most important oilseed crops and is used for the production of high quality edible oil and a feed grade meal. Rapeseed meal has reasonable amino acid but its utilization as a source of protein in human nutrition is limited due to the presence of glucosinolates, phenolic compounds, phytates and hull (Fereidoon & Naezk 1992). The role of these harmful compounds (anti-nutrients) as protective agents against fungi and other pathogens has been reviewed (Butler 1982). Mature grains of mold resistant sorghum cultivars have much higher concentration of flavan - 4 - ols than mold susceptible cultivars (Jambunathan *et al* 1986). The concentration of flavan - 4 - ols in mature sorghum seed should give an indication about the expected reaction of the sorghum cultivars to grain mold in field and could be a very important aid in screening cultivars for grain mold resistance or susceptibility (Jambunathan *et al* 1990). Studies have also shown the major role of phenolic compounds in defence mechanism of plant tissues in response to infections or injuries (Legrand 1983 & Manibhusharaura *et al* 1988). As certain phenolics are bitter tasting and have the ability to precipitate plant and animal proteins, they have been considered as defence compounds against animal predators and microbes (Butler *et al* 1982). Bird resistant sorghum contains condensed tannins (proanthocyanidine, oligomers of flavanols) that are thought to account for their bird repellent properties. The extracted tannin content and the bird repellency of the extracts change considerably during the process of seed maturation and reach a maximum early in the maturation process. The mature seed is usually reported to decline and the decrease in assayable proanthocyanidine of sorghum on maturation has been ascribed to increase polymerization (Bullard *et al* 1981). The decrease in astringency/phenolics is connected with polymerization leading to an increased proportion of higher molecules in fully ripened seeds/fruits (Sattar *et al* 1992). The objective of the present study was to determine the exact stage of maximum synthesis of different phenolics during the leaf-growth of rapeseed for extract preparation to be used as an insect repellent in future.

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Materials and Methods

Three newly evolved species viz. RM-9-7 (*Brassica napus*), BM-1 (*Brassica juncea*) and peela raya *(Brassica carinata)* of rapeseed were sown at the experimental fields using the normal agronomic protocol. The leaves of each cultivar were harvested at 20, 40, 60, 80, 100 and 120 days after sowing for the estimation of various polyphenols. The leaves were washed to remove dust and dirt, and dried with tissue paper. For the estimation of the polyphenols (sinapine, total phenols, procyanidine and leucoanthocyanidine), the samples were extracted in water and methanol. An aqueous extract (1:10, w/v) was prepared by boiling ground sample with water (g / 10 ml) for 30 min. The extract was then filtered and the volume was made up to the required dilution with distilled water. For methanol extract (1:10, w/v) ground tissues were boiled with methanol for 10 min and after decanting off the supernatant liquid, the residue was re-extracted for four successive times

Table 1
Changes in leaf sinapine content (%) of three cultivars of rapeseed

Days after sowing	Methanol extractable				Water extractable			
	1	2	3	Mean	1	2	3	Mean
20	0.206^{l}	0.203^{m}	0.195^{n}	0.201^{F}	0.274^{j}	0.242^{o}	0.329^{e}	0.282^{C}
40	0.221^{j}	0.216^{k}	0.203^{m}	0.213^{E}	0.285^{g}	0.251^{m}	0.356^{a}	0.297^{B}
60	0.268^{c}	0.220^{j}	0.246^{e}	0.245^{B}	0.314^{f}	0.248^{n}	0.274^{j}	0.279^{D}
80	0.291^{a}	0.252^{d}	0.270^{b}	0.271^{A}	0.351^{b}	0.335^{c}	0.332^{d}	0.339^{A}
100	0.246^{e}	0.235^{f}	0.232^{g}	0.238^{C}	0.124^{p}	0.278^{i}	0.280^{h}	0.227^{E}
120	0.223^{i}	0.229^{h}	0.235^{f}	0.229^{D}	0.099^{q}	0.262^{k}	0.258^{l}	0.207^{F}
Mean	0.243^{A}	0.226^{C}	0.230^{B}	-	0.241^{C}	0.269^{B}	0.305^{A}	-

1; RM-9-7 (*Brassica napus*), 2; BM-1 *(Brassica juncea*) and 3; Peela raya (*Brassica carinata*). All observations are average of triplicate readings. Means with same letters are not statistically different ($P < 0.05$).

and all fractions were combined to 100 ml with methanol (Sattar *et al* 1992). All the samples were analyzed for extractable total phenols using Folin-Ciocalteau-phenol reagent, which contains sodium molybdate and sodium tungstate 2.5% and 10%, respectively (Titto 1980). The sinapine and procyanidine content were assayed according to Blair and Reichert (1984). The concentration of sinapine in the methanol extracts was calculated using the formula C = A / EL, where C = concentration in mole/1, A = absorbance at 330 nm, E = extinction coefficient (21390) at 330 nm and L = path length of the spectroscopic cell. The sinapine content was determined by this procedure includes all sinapic acid esters plus free sinapic acid. Procyanidine was determined using HCl / formic acid (1:1) mixture as a complexing reagent. With a solvent of 1-butanol and concentrated HCl, anthocyanidine, formed from flavan - 4 - ols was measured at 550 nm (Dryer *et al* 1981), because flavan - 4 - ols are readily converted to anthocyanidine in acidic solvents at room temperature (Jambunathan *et al* 1986). On heating, the unstable anthocyanidine formed from flavan - 4 - ols are completely destroyed. However, under these conditions flavan - 3 - ol oligomers are converted into anthocyanidine, the resulting absorbance was measured at 550 nm (Subramanian *et al* 1983). The data were subjected to statistical analysis using analysis of variance and the least significant difference (LSD) computed. The means were separated using DMR Test (Steel & Torrie 1980).

Results and Discussion

The major polyphenol of rapeseed is sinapine which constitutes more than 98% of the total phenolic substances (Krygier *et al* 1982; Bibi *et al* 1991; Bibi *et al* 1993). The leaves of variety RM - 9 - 7 contained significantly more mean methanol extractable sinapine than the other two varieties except peela raya where the maximum sinapine contents reached after 40 days as shown in Table 1. The methanol extractable sinapine after 20 days of sowing ranged from 0.195 to 0.206%, which reached to a maximum level of 0.291, 0.252 and 0.270% in variety RM - 9 - 7, BM - 1 and peela raya, respectively after 80 days of sowing. The same trend was followed by water extractable sinapine in all the varieties. After 80 days, the sinapine content (methanol and water extractable) started decreasing and reached minimum after 120 days of sowing. The water extractable sinapine contents were generally more than methanol extractable fraction in all varieties. The interaction analysis showed significant effect ($P < 0.05$) of species and growing days on sinapine contents. Bibi *et al* (1991) found that the varieties and their fractions of tropical rapeseed varied in different phenolic contents in respect of extractants used. The sinapine content ranged from 0.590 to 0.820%, 0.640 to 0.950% and 0.220 to 0.500% in seed, cotyledons and hulls respectively in all varieties. Considerable changes in the assayable amount and bird repellency of tannin during sorghum seed maturation has been reported by Butler (1982). Maximum tannin content reaches in early maturation stages followed by a decline to different levels for different cultivars (Bullard *et al* 1981 & Sattar *et al* 1992).

Total phenol contents (both methanol and water-extractants) reached their maximum after 80 days of growth followed by decline that could be due to increase in polymerization at maturity stage as revealed in Table 2. Among varieties, the leaves of peela raya had a maximum mean total phenols (methanol extractable) followed by BM-1 (0.137%) and RM-9-7 (0.132%). Rapeseed leaves generally contained more methanol extractable total phenols than water extractable and the mean values of total phenols ranged from 0.132 to 0.203%

Table 2
Changes in leaf total phenol content (%) of three cultivars of rapeseed

Days after sowing	Methanol extractable				Water extractable			
	1	2	3	Mean	1	2	3	Mean
20	0.121^{cde}	0.126^{bcde}	0.239^{a}	0.162^{B}	0.021^{fg}	0.005^{g}	0.157^{bc}	0.061^{A}
40	0.153^{bcd}	0.159^{bcd}	0.269^{a}	0.194^{AB}	0.086^{de}	0.031^{efg}	0.188^{b}	0.101^{C}
60	0.161^{bcd}	0.180^{bc}	0.289^{a}	0.210^{A}	0.155^{bc}	0.105^{cd}	0.200^{b}	0.153^{B}
80	0.182^{b}	0.240^{a}	0.257^{a}	0.226^{A}	0.197^{b}	0.259^{a}	0.282^{a}	0.246^{A}
100	0.170^{bc}	0.092^{ef}	0.105^{def}	0.122^{C}	0.076^{def}	N.D	N.D	0.025^{E}
120	0.006^{g}	0.024^{g}	0.235^{fg}	0.030^{D}	N.D	N.D	N.D	-
Mean	0.132^{B}	0.137^{B}	0.203^{A}	-	0.089^{B}	0.067^{C}	0.138^{A}	-

ND; Not determined, 1; RM-9-7 (*Brassica napus*), 2; BM-1 *(Brassica juncea*) and 3; Peela raya (*Brassica carinata*). All observations are average of triplicate readings. Means with same letters are not statistically different ($P < 0.05$).

Table 3
Changes in leaf leucoanthocyanidine (Δ A 550/g) of three cultivars of rapeseed

Days after sowing	Methanol extractable				Water extractable			
	1	2	3	Mean	1	2	3	Mean
20	0.400^{def}	0.360^{ef}	0.180^{g}	0.313	0.150^{gh}	0.380^{d}	0.500^{c}	0.343^{B}
40	0.410^{de}	0.390^{def}	0.227^{fg}	0.342	0.260^{ef}	0.860^{a}	0.600^{b}	0.573^{A}
60	0.440^{de}	1.020^{a}	0.573^{cd}	0.678	0.240^{efg}	0.240^{efg}	0.180^{fgh}	0.220^{C}
80	0.787^{b}	1.000^{a}	0.540^{cde}	0.776	0.300^{de}	0.200^{efgh}	0.100^{hi}	0.200^{C}
100	0.500^{de}	1.110^{a}	0.690^{bc}	0.767	0.220^{efg}	0.140^{gh}	0.140^{gh}	0.167^{D}
120	0.450^{de}	0.990^{a}	0.499^{de}	0.646	0.180^{fgh}	0.020^{i}	0.020^{i}	0.073^{E}
Mean	0.498^{B}	0.812^{A}	0.452^{B}	-	0.225^{B}	0.307^{A}	0.257^{B}	-

1; RM-9-7 (*Brassica napus*), 2; BM-1 *(Brassica juncea*) and 3; Peela raya (*Brassica carinata*). All observations are average of triplicate readings. Means with same letters are not statistically different ($P < 0.05$).

and 0.067 to 0.138%, respectively. A significant increase in polyphenol content of germinated mungbean seeds after 120h has been attributed to fresh synthesis or polymerization of existing polyphenols or degradation of high molecular weight insoluble polymers into smaller molecular weight soluble polymers that give colour to the estimating reagent (Charlene *et al* 1985). The total phenol concentration in apples is reported to stay at relatively constant level during storage (Coseteng & Lee 1987), whereas sinapine, catechin, total phenols and leucoanthocyanidine in persimmon have been reported to change in concentration during storage (Bibi *et al* 2001) and solar drying (Chaudry *et al* 1998).

The leucoanthocyanidine were more extractable in methanol ranging from 0.400 to 0.787, 0.360 to 1.110 and 0.180 to 0.690 Δ A 550 /g in RM - 9 - 7. Peela raya and BM-1 leaves as compared to water extractable with values ranging from 0.150 to 0.300, 0.380 to 0.860 and 0.343 to 0.573 ΔA 550 /g for leaves of same varieties, respectively, during 120 days of growth period as reported in Table 3. The leucoanthocyanidine contents were maximum (0.79 Δ A 550 /g) after 80 days of sowing in RM - 9 - 7 leaves while for varieties peela raya and BM-1, the highest values of 1.110 and 0.690 Δ A 550 /g, respectively after 100 days of sowing. In case of water extractant, the leucoanthocyanidine content reached its maximum value of 0.3 Δ A 550 /g after 80 days of sowing for RM-9-7 leaves while for varieties peela raya and BM-1, the leucoanthocyanidine contents were maximum (0.86 and 0.60 Δ A 550 /g, respectively) after 40 days of sowing followed by decline during further growth. For all the three varieties, the methanol extractable procyanidine content were 0.025 - 2.570, 0.140 - 4.140 and 0.160 - 2.460 ΔA 550 /g as compared to water soluble procyanidine content with values of 0.060 - 0.620, 0.120 - 1.520 and 0.086 - 0.280 ΔA 550 /g for RM - 9- 7, peela raya and BM-1 leaves, respectively. In case

Table 4
Changes in leaf procyanidine (Δ A 550/g) in three cultivars of rapeseed

Days after sowing	Methanol extractable				Water extractable			
	1	2	3	Mean	1	2	3	Mean
20	0.02430^{hi}	0.01367^{i}	0.1567^{gh}	0.649	0.0583^{l}	0.1200^{jk}	0.0799^{kl}	0.086^{E}
40	0.1833^{hi}	0.2143^{fg}	0.3000^{f}	0.196	0.1220^{jk}	0.1843^{i}	0.1200^{jk}	0.142^{D}
60	2.520^{c}	3.687^{b}	2.227^{d}	2.811	0.0797^{kl}	0.5833^{f}	0.1600^{ij}	0.274^{C}
80	2.467^{c}	4.080^{a}	2.420^{c}	2.989	0.6200^{f}	1.100^{c}	0.2867^{h}	0.669^{B}
100	1.853^{e}	2.287^{d}	1.883^{e}	2.008	0.2100^{i}	1.523^{a}	0.7867^{d}	0.840^{A}
120	1.823^{e}	2.443^{c}	1.790^{e}	2.019	0.4000^{g}	1.341^{b}	0.7067^{e}	0.816^{A}
Mean	1.460^{B}	2.121^{A}	1.463^{B}	-	0.248^{C}	0.808^{A}	0.357^{B}	-

1; RM-9-7 (*Brassica napus*), 2; BM-1 *(Brassica juncea*) and 3; Peela raya (*Brassica carinata*). All observations are average of triplicate readings. Means with same letters are not statistically different ($P < 0.05$).

of methanol soluble procyanidine content, the leaves of RM - 9 - 7 had maximum value of 2.57 ΔA 550 /g after 60 days of sowing, while leaves of peela raya and BM-1 showed maximum values of 4.14 and 2.46 (Δ A 550 /g), respectively after 80 days of sowing followed by decrease upto 120 days. The water soluble procyanidine content of RM-9-7 leaves showed maximum value of 0.62 Δ A 550 /g after 80 days of sowing and for leaves of peela raya and BM-1, the highest values were 1.52 and 0.80 Δ A 550 /g, respectively after 100 days of sowing followed by decrease in procyanidine content for all the leaves of the three varities due to some interconversion of the phenolic compounds during further growing period Table 4.

It has been reported that differences existed in the quantities of condensed tannins including procyanidine and simple phenols both as function of maturation and cultivar in peaches (Samuel and Callakan 1990). Deposition of these compounds began in the early stages of development in all cultivars evaluated and increased to maximize between the first and second swell in fruit growth (Amiot *et al* 1992). This is followed by diminution of phenolics during ripening and the greatest differences seen between cultivars were those related to oleuropin and verbascoside.

Conclusion

It can be concluded from the results that maximum phenolics content were found after 80 days. However, a generalization regarding the number of days to maximize phenolics contents of all kind and all applicable extractants and for all the species of rapeseed may not be possible as in some cases different phenolics compounds are maximum at different growth stages in different species and different extractants. The character seems to be genetically controlled and for each species it will have to be determined separately.

Acknowledgement

The authors are thankful to the Director, NIFA for providing facilities of the present research work.

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