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ANTICARCINOGENIC APPROACH TO CONSERVATION OF RICE LANDRACES OF GANGETIC ALLUVIAL ZONE

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Abstract

The coloured rice is known to have antioxidant property due to presence of flavenoid and anthocyanin having powerful activity of scavenging of free radicals than other white rice. Recent focus on the study of agriculture is to develop a promising variety with quality trait and specially having mineral contents along with antioxidant properties for mitigating the arising problem of diabetes and cancer. In any varietal improvement planning, these wild or landraces provide a valuable gene pool to develop a promising line which may have resistant power to fight against any environmental stress like pest and diseases. Keeping this in view, the present investigation aims at finding out and characterizing these landraces from Farmer's field focusing on their presence of anthocyanin in their various body parts and their conservation for future plant breeding programme so that the genes for anthocyanin i.e., having antioxidant properties which may be incorporated into our present high yielding varieties using various biotechnological approach. Also it is suggested to implement and advice in daily intake of this coloured rice to keep away diabetes and cancer from this world.

Keywords: anthocyanin colouration, rice landraces, scavenger, conservation

Introduction

The rice growing regions of alluvial zone of West Bengal include a range of diverse agro-ecological niches with a number of diverse lines (Chakravorty and Ghosh, 2013, 2014, 2015; Chakravorty *et al.*, 2014). Though once (before 1970's) all the rice fields of Bengal were occupied by a number of traditional lines, in the post-green revolution period *i.e.* starting from early seventies to till date, a good number of such lines were replaced by high yielding rice lines (Richharia 1979). Genetic variability studies are important in selection of parents for hybridization (Chaudhury and Singh, 1982) as sound crop improvement depends upon the magnitude of variability in the base population (Adebisi *et al.*, 2001). Genetic variability ensures crop improvement through the use of appropriate selection methods.

About Anthocyanin

Anthocyanins are a class of compounds belonging to the larger flavonoids class which comprises a subset of the polyphenol class of compounds. The polyphenol class of compounds includes all molecules with more than one hydroxyl group on an aromatic ring. Flavonoids compounds share a common framework consisting of two aromatic rings (A and B), that are bound together by three carbon atoms forming an oxygenated heterocycle (ring C). Flavonoids are subsequently divided into several groups differing in the oxidation state of the heterocyclic pyran ring C. The subclasses consist of flavanols, flavanones, flavones, isoflavones, flavonols, and anthocyanins (listed in ascending order of oxidation) and within each of these subclasses, individual compounds are characterized by specific hydroxylation and conjugation patterns (Beecher, 2003). Most flavonoids are present in nature as the glycosidic form, with the exception of flavanols, and this contributes to their complexity and the large number of individual molecules that have been identified (Harborne et al., 2000). Considering all these factors, the number of probable anthocyanin compounds is quite large, leading to over 600 having been identified from natural sources (Delago et al., 2000). Anthocyanins are responsible for much of the red, blue, and purple colors of fruits, vegetables, grains, flowers, and herbs, which explains their name, in Greek, *anthos* means flower and *kyanos* means blue. Anthocyanins are almost exclusively found in higher plants, although a few have been found in lower plants such as mosses and ferns (Delago et al, 2000; Fossen et al, 2002. In general, the anthocyanins in most of the fruits and vegetables are observed in concentrations from 0.1% up to 1.0% dry weight Delago, 2000; Mazza and Miniati, 1993). Anthocyanins are included in the list of natural compounds known to work as powerful antioxidants. In the last years, great attention was given to the possible protection exerted by natural antioxidants present in dietary plants, towards tissue injury mediated by reactive oxygen species (ROS).

Apoptosis and role of anthocyanin

The daily intake of anthocyanins in humans has been estimated at 180-215 mg/d in USA(Kubnau, 1976) Numerous studies have

shown that anthocyanins display a wide range of biological activities (Mazza et al., 2000) including antioxidant (Mazza et al., 2002; Wang et al., 1997; Tsuda et al., 1998) anti-inflammatory (Wang and Mazza, 2000), antimicrobial (Pisha and Pezzuto, 1994) anti-carcinogenic and proapoptotic activities (Kamei et. Al, 1995; Katsube et al., 2003; Kang,et al., 2003) improvement of vision (Mercier et al., 1965; Matsumoto et al., 2003) and neuroprotective effects. In addition, anthocyanins display a variety of effects on blood vessels (Andriambeloson et al., 1998) and platelets (Morazzoni et al., 1990; Demrow et al., 1995) that may reduce the risk of coronary heart disease. Keeping this in view, this study was framed to collect , characterize and conserve those promising rice landraces of Bengal Basin having anthocyanin coloration in their different body parts so taht they may be used and conserved to use them to remove cancer and related hazards from the nation through intake of food.

Materials and Methods

A total of 51 rice cultivars from Zonal Adaptive Research Station, Krishnagar were used for mineral content analysis. These cultivars were developed in a randomized complete block design with three replications at the research farm of Zonal Adaptive Research Station, (23˚24'N latitude and 88˚31'E longitude with an altitude of 9.75 meters above mean sea level) Krishnagar, Nadia, West Bengal, India during kharif season for three consecutive years of 2010, 2011 and 2012. The soil was slightly acidic with pH of 6.0, low soluble salts (EC of 0.15 dS m^{-1}**),** medium organic carbon content (0.57%), Total N (0.056%), medium in available P (25.28 kg ha^{-1}) and K (148.77 kg ha^{-1}). The experimental site belongs to tropical humid climate having the average rainfall of 1464 mm, most of the amount falls in between June to September. The minimum temperature reaches 7.6˚C in the month of January and the maximum 41.1˚C in the month of May. It has been observed that 74.7% of the annual rainfall is obtained during June to September and more than 83.6% during June to October. The materials were transplanted in 3.0×2.85m^2 plot with plant to plant spacing 15 cm within a row and row to row spacing of 20 cm. plot to plot distance was 60 cm. A random sample of five competitive plants was used for observations on different traits under study. Crop was raised following recommended package of practices. Fertilizers ($N:P_2O_5:K_2O$) @ 50:25:25 kg ha^{-1} were applied. The observation of various characteristics was recorded at different stages of growth with appropriate procedures as per the DUS test guidelines of PPV & FR Act, 2001.

Results and Discussions

Maximum cultivars showed the significant variability in respect to anthocyanin coloration in their different body parts including different colours in seeds. It is known that purple rice contains the dark purple pigments in its bran (outer coat of the rice), which are mainly anthocyanins. The anthocyanin-rich extract from black rice (AEBR) reduces the viability of breast cancer cell lines MCF-7 (ER(+), HER2/neu(-)), MDA-MB-231 (ER(-), HER2/ neu(-)), and MDA-MB-453 (ER(-), HER2/neu(+)) and induces apoptosis in MDA-MB-453 cells via the intrinsic pathway *in vitro* by activating caspase cascade, cleaving poly (ADP-ribose) polymerase (PARP), depolarizing mitochondrial membrane potential, and releasing cytochrome c. Oral administration of AEBR (100 mg/ kg/day) to BALB/c nude mice bearing MDA-MB-453 cell xenografts significantly suppresses tumor growth and angiogenesis by inhibiting the expression of angiogenesis factors MMP-9, MMP-2, and uPA in tumor tissue (Hui et al., 2010).

The ethyl acetate extract of "Kurosu" (EK), Japanese traditional vinegar from unpolished rice, inhibits cell proliferation of human cancer cell lines, viz., colon adenocarcinoma (Caco-2), lung carcinoma (A549), breast adenocarcinoma (MCF-7), bladder carcinoma (5637), and prostate carcinoma (LNCaP) cells. Flow cytometry of EK-treated Caco-2 cells demonstrates a decrease of cell number in the G(2)/M phase and an increase in the sub-G1 phase (apoptotic). p21 mRNA expression is induced in EK-treated Caco-2 cells. Thus, EK causes G0/G1 arrest through p21 induction in Caco-2 cells (Nanda et al., 2004).

Anticarcinogenic activity

Anthocyanins inhibit metastasis through regulation of MMP-2 and MMP-9 also in B16-F1 cells, and it modulates the expression levels of Ras, PI3K, phospho-Akt, and NF-κB (Huang et al., 2008) In breast cancer, treatment of HGF-stimulated MCF-10A cells with delphinidin decreased the expression of Met and the phosphorylation of FAK and Src; induction of the paxillin, Gab-1, GRB-2, Ras–ERK MAPKs, and PI3K/Akt/ mTOR/p70S6K pathways was also inactivated by delphinidin. Moreover, the blockage of HGF-mediated activation of NF-kB and STAT3 and translocation of PKC were also observed in delphinidin-treated MCF-10A cells (Syede et al.,2008). Cyanidin 3-glucoside attenuated ethanol-induced migration, invasion, and cell–matrix adhesion and inhibited ethanol stimulated phosphorylation of ErbB2, cSrc, FAK,

and p130Cas of high ErbB2-expressing breast cancer BT474, MDA-MB-231, and MCF-7ErbB2 cells (Xu et al.,2010). In prostate cancer, anthocyanin-enriched fractions from blueberry (*Vaccinium angustifolium*) down regulate MMP-2/MMP-9 and upregulate TIMP-1/TIMP-2 in DU145 cells by modulating PKC and MAPK pathways (Matchett et al., 2005; Matchett et al., 2006) The inhibitory effects of anthocyanins on motility and invasion of HCT-116 human colon carcinoma cells were associated with the suppression of claudin (a tight junction-related protein) and the inhibition of MMP-2/MMP-9 through p38MAPK and PI3K/Akt pathways (Shin *et al.*,2011). In oral and cervical cancer, the invasion of SCC-4 cells and HeLa cells were diminished by the treatment of peonidin 3-glucoside and cyaniding 3-glucoside. In fibrosarcoma, delphinidin slightly inhibited the activities of MMP-2/MMP-9, which might responsible, in part, for the inhibition of invasion in HT-1080 cells. In glioblastoma, Lamy (Lamy *et al.*, 2007) revealed that the aglycons of the most abundant anthocyanins in fruits, including cyanidin, delphinidin, and petunidin, act as potent inhibitors for the migration of glioblastoma U-87 cells.

Our Experience

Considerable variability and diversity in respect to quantitative traits have been found among some elite landraces of rice of West Bengal (Chakravorty and Ghosh, 2013; Chakravorty *et al.,* 2013). Qualitative traits were studied following DUS test and out of 51 cultivars, 27 cultivars were found to have anthocyanin in their different body parts (Table 1).

Table 1 : Twenty seven cultivars showed the presence of anthocyanin colouration from DUS Test

Code	Name of the cultivars	Anthocyanin colouration in					
		Auricle	Auricle & Leaf collar	Ligule	Lemma	Leaf Sheath	Stem
G2	Badhabna					+	
G5	Dhuladhan		+		+		+
G6	Dhuri			+			+
G7	Kalamkathi(W)			+			
G8	Suakalama						+
G17	Dudhkalama	+			+		
G20	Agnisal					+	
G21	Chadrakanta	+	+	+			
G22	Muktasal				+		+
G23	Punjabsal			+	+		
G24	Sitasal	+					
G27	Laldhusri					+	
G28	Malliksal					+	
G29	Baidjhulur					+	
G30	Jhulur		+				
G33	Danaguri						+
G36	Netaisal					+	
G37	Sankar kalma			+			
G38	Rupsal				+		
G39	Jhingasal	+					
G41	Jhuli	+	+				
G42	Raja Badsa						+
G45	Kerala Sundari						+
G46	Balaram sal		+				
G49	Lalhusri						+
G50	Annanda				+		
G51	Sarkele(aman)	+	+	+	+		+

+ indicates the presence of anthocyanin coloration

Agro-biodiversity and Hot-spots (Section 45 of PPV & FR Act, 2001 read with rule 70 of PPV 7 FR rules, 2003)

Much of the agricultural biodiversity is being lost from its natural habitats due to expansion of agricultural production activities to frontier areas and also from the agricultural fields due to adoption of improved varieties and other unsustainable technologies by the farmers. Agricultural biodiversity acts as a safety net against varieties of nature and man-made disasters. It also partially insulates the mankind from the probable holocaust in the event of threat to one or two crops or plant species. Conservation of agro-biodiversity is, in any case, required to sustain food production for ever increasing population and is also required to maintain ecological balance in the ecosystems. To define and demarcate the areas which are to be identified as Agro-biodiversity hotspots, before the support and rewards can be framed for farmers or community of farmers, PPV & FR

Authority constituted a Task Force which after several rounds of discussions at different levels submitted its report which was published in two volumes book which have been widely distributed for creating awareness.

Conclusion

So, with reference to above discussion, it may be definitely said that we should conserve the traditional rice landraces having natural coloured plant parts including seed and to include in our daily food intake to keep away the fatal diseases and to save the nation from different health hazards including cancer

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