Explicating genetic architecture governing nutritional quality in pigmented rice

Jebi Sudan [1], Uneeb Urwat [1], Asmat Farooq [1], Mohammad Maqbool Pakhtoon [1], Aaqif Zaffar [2], Zafir Ahmad Naik [2], Aneesa Batool [1], Saika Bashir [1], Madeeha Mansoor [1], Parvaze A. Sofi [2], Najeebul Rehman Sofi [3], Asif B. Shikari [2], Mohd. Kamran Khan [4], Mohammad Anwar Hossain [Corresp., 5], Robert J. Henry [6], Sajad Majeed Zargar [Corresp. 1]

[1] Proteomics Lab., Division of Plant Biotechnology, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, Srinagar, Jammu and Kashmir, India

[2] Division of Genetics and Plant Breeding, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir (J&K), Srinagar, Jammu and Kashmir, India

[3] Mountain Research Centre for Field Crops, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, Khudwani, Jammu and Kashmir, India

[4] Department of Soil Sciences and Plant Nutrition, Faculty of Agriculture, Selcuk University, Konya, Turkey

[5] Genetics and Plant Breeding, Bangladesh Agricultural University, Mymensingh, Bangladesh

[6] Queensland Alliance for Agriculture and Food Innovation, Queensland University, Brisbane, Australia

Corresponding Authors: Mohammad Anwar Hossain, Sajad Majeed Zargar
Email address: anwargpb@bau.edu.bd, smzargar@skuastkashmir.ac.in

Rice is one of the most important staple plant foods that provide a major source of calories and nutrients for tackling the global hunger index especially in developing countries. In terms of nutritional profile, pigmented rice grains are favoured for their nutritional and health benefits. The pigmented rice varieties are rich sources of flavonoids, anthocyanin and proanthocyanidin that can be readily incorporated into diets to help address various lifestyle diseases. However, the cultivation of pigmented rice is limited due to low productivity and unfavourable cooking qualities. With the advances in genome sequencing, molecular breeding, gene expression analysis and multi-omics approaches, various attempts have been made to explore the genetic architecture of rice grain pigmentation. In this review, we have compiled the current state of knowledge of the genetic architecture and nutritional value of pigmentation in rice based upon the available experimental evidence. Future research areas that can help to deepen our understanding and help in harnessing the economic and health benefits of pigmented rice are also explored.

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5 Robert J Henry[6] and Sajad Majeed Zargar[1*]

6 [1]Proteomics Lab., Division of Plant Biotechnology, Sher-e-Kashmir University of Agricultural
7 Sciences and Technology of Kashmir, Srinagar, Jammu and Kashmir, India
8
9 [2]Division of Genetics and Plant Breeding, Sher-e-Kashmir University of Agricultural Sciences
10 and Technology of Kashmir, Srinagar, Jammu and Kashmir, India
11
12 [3]Mountain Research Centre for Field Crops, Sher-e-Kashmir University of Agricultural Sciences
13 and Technology of Kashmir, Khudwani, Jammu and Kashmir, India
14
15 [4]Department of Soil Sciences and Plant Nutrition, Faculty of Agriculture, Selcuk University,
16 Konya 42079, Turkey
17
18 [5]Department of Genetics and Plant Breeding, Bangladesh Agricultural University, Mymensingh
19 2202, Bangladesh
20
21 [6]Queensland Alliance for Agriculture and Food Innovation, University of Queensland, Brisbane,
22 Qld, 4072, Australia
23
24 [#]corresponding authors: smzargar@skuastkashmir.ac.in; anwargpb@bau.edu.bd
25
26

27 **Abstract**

28 Rice is one of the most important staple plant foods that provide a major source of calories and
29 nutrients for tackling the global hunger index especially in developing countries. In terms of
30 nutritional profile, pigmented rice grains are favoured for their nutritional and health benefits.
31 The pigmented rice varieties are rich sources of flavonoids, anthocyanin and proanthocyanidin
32 that can be readily incorporated into diets to help address various lifestyle diseases. However, the
33 cultivation of pigmented rice is limited due to low productivity and unfavourable cooking
34 qualities. With the advances in genome sequencing, molecular breeding, gene expression
35 analysis and multi-omics approaches, various attempts have been made to explore the genetic
36 architecture of rice grain pigmentation. In this review, we have compiled the current state of
37 knowledge of the genetic architecture and nutritional value of pigmentation in rice based upon

38 the available experimental evidence. Future research areas that can help to deepen our

39 understanding and help in harnessing the economic and health benefits of pigmented rice are also

40 explored.

41 **Keywords:** Pigmented rice, nutrients, secondary compounds, breeding, genetic engineering

42

43 **Introduction**

44 The sustainable food systems for ever-increasing human population include not only adequate

45 food but also adequately balanced nutrient rich food. An increased focus on foods that provide

46 adequate nutrition has resulted from a deeper understanding of the role of nutrition in maintaining

47 a healthy population, particularly in developing nations.

48 Rice is an important staple crop that feeds more than half of the world's population and is

49 being cultivated on approximately 158 million hectares of land producing around 850 million

50 tons of grains annually (*Krishnan et al., 2020*). Asia represents a major rice growing region

51 amounting for about 85% of the total production, followed by Latin America and Africa. India,

52 being the second largest producer of rice in the world contributes around134 million metric

53 tonnes with productivity of 2.8 t/ha and in a total cultivated area of around 46 million hectares

54 (https://www.statista.com/statistics/agriculture/farming/2022). Rice is a good option for a healthy

55 diet as it has no cholesterol, fat, or sodium and contains eight essential amino acids in a balanced

56 proportion. Rice bran oil is rich in linoleic and oleic acid, which are essential for sustaining cell

57 membranes and nervous system functioning (*Bhat et al., 2020*). Rice grains come in a variety of

58 pigmentations, including yellow, green, brown, red, purple, and black. Pigmented rice (or

59 coloured rice) has long been considered to have nutraceutical benefits resulting in ongoing

60 production as niche rice in various parts of the world.

61 Pigmented landraces of rice have a higher content of total anthocyanin, total phenol and

62 polyphenol which signifies high antioxidant potential (*Deng et al., 2013*). Due to their rich

63 nutritional profile and high antioxidants, these rice types have the potential to boost human health

64 by addressing a variety of metabolic disorders (**Figure 1**). The consumption of coloured rice

65 reduces oxidative stress while simultaneously increasing antioxidant capacity in animal models,

66 and this may be linked to a lower risk of chronic diseases like cardiovascular disease, type 2

67 diabetes and some cancers (*Wongsa, 2020*). As a result of their health potential and widespread

68 demand, several nations have evaluated their coloured rice and created newer types. Varieties of

69 coloured rice, recognized for their nutritional advantages, have remained a primary crop in

70 several regions of India (**Figure 1**). Molecular breeding and biotechnology approaches are

71 regularly used for increasing the nutritional components in pigmented rice as well as in

72 transferring these quality traits to conventional white rice. However, there lies a gap between the

73 research that is available in the form of specific publications and the thoughtful compiled

74 information that is required to gain an overall understanding of the topic. Hence, the present

75 review highlights information on the important nutritional components in pigmented rice grains

76 along with the efforts made to increase the nutritional quality of both pigmented and white rice

77 through various biotechnological approaches to address nutritional food security. The present

78 review will serve as a comprehensive review for scholars, scientists and students working in the

79 area of pigmented rice.

80 **Survey Methodology**

81 For this article, we have conducted a rigorous literature review and collected information from

82 research articles, review articles, book chapters, websites (www.statista.com/;

83 www.agricoop.gov.in/) and databases (http://rice.uga.edu/; https://www.gramene.org/;

84 https://rapdb.dna.affrc.go.jp/). We have also used Google image for drawing a map of India. We

85 excluded the studies having abstracts available only with no full-text articles

86 **Nutritional Composition of Pigmented Rice**

87 *Protein and Amino acids*

88 Our bodies' healthy condition as well as tissue repair depends on the protein that we take from

89 foods. Since rice is a staple food for a major part of the global population so rice protein is

90 important in human nutrition. Rice protein concentrates and isolates are useful components in

91 many food applications because they offer special nutritional qualities that set them apart from

92 other cereals and legume proteins, such as a well-balanced amino acid profile and being easily

93 digestible and non-allergic (*Romero,* 2014; *Sati and Singh, 2019*). Rice proteins are also

94 excellent ingredients for baby food recipes due to their distinct nutritional and hypoallergenic

95 qualities. Rice protein hydrolysates have the potential to improve food emulsion stability and

96 serve as a natural antioxidant (*Rani et al., 2018*). In rice, glutelins dominates seed storage

97   proteins, making up to 60–80% of the total protein in the seed by weight. Compared to other
98   grain seeds, that contains higher prolamine content as a proportion of total protein, they make up
99   only roughly 20–30% of the protein in rice seeds (*Kawakatsu et al., 2008*).

100   Moreover, the amount and quality of protein is much better in pigmented rice as
101   compared to non-pigmented rice. *Chen et al. (2016)* compared pigmented and white rice grains
102   at five different stages of growth and found 230 differently abundant proteins associated with
103   several metabolic activities. The pigmented grains were shown to have lower concentrations of
104   proteins involved in signalling pathways, redox homeostasis, photosynthesis, nitrogen fixation,
105   and tocopherol synthesis. In contrast, it was discovered that the pigmented grain had higher
106   levels of proteins necessary for the synthesis of sugars and flavonoids. Pigmented rices were also
107   found to have a higher concentration of proteinogenic amino acids (histidine, threonine, valine,
108   iso-leucine, methionine, phenyl alanine, lysine, proline, and tyrosine) and non-proteinogenic
109   amino acids (glutamic acid, aspartic acid, asparagine, citrulline, and GABA) as compared to non-
110   pigmented rice (*Samyor et al., 2017*; *Kaur et al., 2018*).

111   Genetic engineering technology has substantially helped in improving cultivated crops
112   through improvement of their essential amino acid and protein content. To raise the lysine
113   content in cereals, lysine may be added at alternative codons during translation using a
114   recombinant tRNA-lysine. Recombinant production of this tRNA in transgenic rice could greatly
115   increase the amount of lysine in the seeds (*Wu and Chen, 2003*). *Liu et al. (2016)* used an
116   endosperm-specific promoter for expression of the lysine-rich protein (LRP) gene from
117   *Psophocarpus tetragonolobus* that results in 30% more lysine in the transgenic rice seeds as
118   compared to wild-type plants. Lysine biofortification in rice seeds was also achieveded by over-
119   expressing two endogenous lysine-rich histone proteins (RLRH1 and RLRH2) *(Wong et al.*
120   *2015)*. Moreover, it was also found that increased lysine content also increases the threonine
121   content in the grains through direct regulation (*Das et al., 2020*). The quantity of free tryptophan
122   in the seeds increased significantly as a result of expression of an equivalent feedback-insensitive
123   α-subunit of the rice anthranilate synthase, but other important agronomical variables, including
124   spikelet fertility, yield, and germination, are negatively correlated (*Wakasa et al., 2006*).

125   *Phytosterols, Carotenoids and Vitamins*

126   Plant sterols and plant stanols are collectively referred to as "phytosterols". The natural

127   phytosterol dietary intake varies from 150 to 450 mg per day, depending on a person's eating

128   habits (*Ostlund Jr, 2002*). Phytosterols have many beneficial effects such as anti-cancer activity,

129   lowering blood levels of negative lipoproteins and cholesterol absorption (*Schaefer, 2002*;

130   *Suttuarporn et al., 2015*). The three main and prevalent phytosterols found in human diet are β-

131   sitosterol, campesterol, and stigmasterol. β-sitosterol is the most common phystosterol found in

132   commercial rice cultivars, followed by campesterol, 15-avenasterol, and stigmasterol (*Zubair et*

133   *al., 2012*). Three other sterols, including fucosterol, 24-methylene-ergosta-5-en-3b-ol, and 24-

134   methylene-ergosta-7-en-3b-ol, are also present in the bran of the black rice variety "Riceberry"

135   (*Suttiarporn et al., 2015*).

136   γ-oryzanol, is another class of phytosterols and is made up of a variety of phytosteryl

137   ferulates, notably cycloartenyl ferulate, 24-methylenecycloartanyl ferulate, b-sitosteryl ferulate,

138   and campesteryl ferulate. γ-oryzanol seems to be accumulated at a faster rate in pigmented grain

139   compared to non-pigmented grain (*Chakuton et al., 2012*). Moreover, its value is found to be

140   higher in black and purple rice as compared to red rice in Thai cultivars (*Sornkhwan et al.,*

141   *2022*).

142   The essential isoprenoid phytonutrients known as carotenoids, are produced in plastids,

143   and are known to be lacking in rice endosperm. Provitamin A, found in carotenoids, are also

144   components that lower the risk of a number of diseases like cancer, heart disease, age-related

145   muscular degeneration, immune system disorders, and certain other degenerative diseases

146   (*Perera and Yen, 2007*; *Bollineni et al., 2014*). Lutein and zeaxanthin account for more than 90%

147   of the carotenoids synthesised in rice but trace levels of other carotenes such as lycopenes and

148   beta-carotene are also present (*Pereira-Caro et al., 2013*; *Melini and Acquistucci, 2017*). Most of

149   these compounds are found in the bran, with milled rice containing little or no carotenoids

150   (*Petroni et al., 2017*). The genetically variable feature of grain carotenoid concentration is highly

151   associated with grain colour. White rice have extremely little carotenoid content, but red and

152   black grains have much more (*Ashraf et al., 2017*; *Petroni et al., 2017*).

153   Vitamins constitute an important component of a balanced diet. Both tocopherols and

154   tocotrienols (enriched sources of vitamin E) arefound in rice grains (*Zubair et al., 2012*). The

155   most prevalent tocotrienols in rice are the β- and γ-tocotrienols (*Irakli et al., 2016*). According to

156   *Gunaratne et al. (2013),* red rice grains contain higher total levels of tocopherol and tocotrienol

157 compared to white rice varieties. The quantity of tocopherol in the grain is, however,

158 dramatically decreased by the processes of dehulling and milling (*Zubair et al., 2012*). Rice is

159 deficient in vitamins A, C and D. However, brown rice has been found to contain significant

160 amounts of vitamin B complex {thiamin (B1), riboflavin (B2), niacin (B3), pantothenic acid

161 (B5), pyridoxine (B6), biotin (B7), folate (B9)} and E (α-tocopherol) (*Juliano, 1985; Samyor et*

162 *al., 2017*). Through molecular biology and genomic techniques, tremendous progress has been

163 achieved in the genetic engineering of carotenoid production in plants during the past few

164 decades. The whole collection of carotenoid biosynthesis pathway genes and related enzymes

165 have been described. Metabolic engineering of the carotenoid biosynthesis pathway was carried

166 out by marker assisted backcross breeding of two genes- phytoene synthase (*Zmpsy1*) from *Zea*

167 *mays* and carotene desaturase (*Crtl*) from common soil bacterium *Pantoea ananatis* to create

168 second generation transgenic rice, which accumulates phytoene, a crucial provitamin A

169 intermediate (*Mallikarjuna et al., 2021*; *Biswas et al., 2021*). Due to their instability and

170 degradation over time in long-term storage, folates in rice grain are less available. By genetic

171 engineering a folate-binding protein, which increases the stability of folates by attaching to it

172 over prolonged storage periods, biofortified high-folate rice grains were created that had 150

173 times more folate than wild rice (*Blancquaert et al., 2015*).

174   The mutant TNG71-GE brown rice variety was found to be richer in total tocopherol and

175 tocotrienol than the wild-type. As a result, this mutant TNG71-GE rice variety could be used to

176 produce a crop with high vitamin E content (*Jeng et al., 2012*). Later, it was also found that a

177 single-point mutation of the giant embryo gene (*GE*) in Chao2-10 rice led to the development of

178 a new mutant known as Shangshida No. 5. When compared to Chao2-10, Shangshida No. 5

179 brown rice contains more total vitamin E and α-tocopherol (*Wang et al., 2013*). The overall

180 amount of tocochromanols in rice was very slightly enhanced in transgenic rice created by

181 constitutive overexpression of the *Arabidopsis thaliana* r-hydroxyphenylpyruvate dioxygenase

182 (*HPPD*) gene (*Farre et al., 2012*). The elite Japanese rice cultivar Wuyujing 3 (WY3) provides

183 transgenic brown rice with increased quantities of α-tocotrienol by both constitutive and

184 endosperm-specific over expression of the *Arabidopsis g-TMT (AtTMT)* gene (*Zhang et al.,*

185 *2013*).

186 *Flavonoids*

187   Plants contain large amounts of flavonoids, which are secondary metabolites and play a

188   significant role in plant development, pigmentation, UV protection, as well as in safeguarding

189   the interaction with microorganisms (*Samanta et al., 2011*). Coloured flavonoids (flavanols,

190   isoflavonoids, and flavones) are the pigments responsible for the colour of leaves, fruits, and

191   flowers (*Yang et al., 2022*). Flavonoids play a crucial role in floral colours and fragrance, fruit

192   pollinator attraction, and fruit dispersion (*Panche et al., 2016*). Due to the presence of

193   flavonoids, terpenoids, steroids, and alkaloids, pigmented rice exhibits cytotoxic, anti-tumor,

194   anti-inflammatory, antioxidant, and neuroprotective activities (*Goufo and Trindade, 2014*). In a

195   recent study on thetotal flavonoid content (TFC) of rice, it was found that black and red rice had

196   a higher TFC when compared to white rice varieties (*Chen et al., 2022*). Based on the amount of

197   aromatic compounds, flavonoids can be classified into a wide range of groups such as chalcones,

198   pro anthocyanidins, anthocyanins, flavones, flavonols, flavanones, and flavanonols (*Mbanjo et

199   al., 2020*). However, proanthocyanidins and anthocyanins are the two primary flavonoids present

200   in pigmented rice. Anthocyanins are responsible for the purple to blue coloration of such grains

201   (*Zhang et al., 2015*). Consuming foods high in these substances may reduce inflammation and

202   lower the risk of developing type-2 diabetes, cancer, and heart disease (*Rengasamy et al., 2019*).

203   Eating foods high in anthocyanins on a regular basis also enhanced memory and overall brain

204   health (*Henriques et al., 2020*).

205   Red and white rice grains lack anthocyanin (*Xiongsiyee et al., 2018*), although some red

206   and brown rice accessions have low levels (*Ghasemzadeh et al., 2018*). The enzyme anthocyanin

207   reductase converts unstable anthocyanidins into the colourless flavan-3-ols epiafzelechin,

208   epicatechin, and epigallocatechin, and when these molecules are glycosylated, a broad variety of

209   unique compounds are produced (*Kim et al., 2015*). After examining the transcription of eight

210   flavonoid biosynthesis genes in various rice cultivars, it was found that pigmented variants had

211   stronger expression of genes than non-pigmented forms (*Mbanjo et al., 2020*). At least two

212   chalcone synthetase-encoding genes *CHS2* on chromosome 7 and *CHS1* on chromosome 11,

213   help the production of flavanones in rice (*Cheng et al., 2014*). Proanthocyanidins are produced

214   by three flavone 3-hydroxylases: *F3H-1* (on chromosome 4), *F3H-2* (on chromosome 10), and

215   *F3H-3* (on chromosome 4) (*Park et al., 2016*). An effective method for researching genetic

216   variants and genetic engineering in plants is the recombinant technology (*Mackon et al., 2021*).

217   Understanding the function of several genes associated with anthocyanin biosynthesis and the

218   development of anthocyanin in the endosperm has become possible due to analysis of the

219   anthocyanin colouring mechanism in rice. The regulator of a rice prolamin gene was used to

220   insert the maize *C1/R-S* regulatory genes into the white rice japonica cultivar Hwa-Young that

221   resulted in the production of a wide range of flavonoid compounds (*Mackon et al., 2021*).

222   *Phenolics*

223   Polyphenols constitute the most common secondary metabolites of plants and seem to be largely

224   indigenous to the plant kingdom (*Dai et al., 2010*). They are essential for the plant's

225   development, fertilization, and defence against viruses, parasites, and environmental conditions

226   including light, cold, pollutants, and also impact the plant's colour (Kabera *et al., 2014*). Phenolic

227   compounds benefit humans by reducing the risk of contracting chronic diseases, have a high

228   antioxidant property and make significant contributions to the prevention of many oxidative

229   stress-related diseases, including malignancy. Furthermore, there has been a lot of focus on

230   identifying and synthesising phenolic compounds or extracts from diverse plants in the realms of

231   health care and medicine (*Dai et al., 2010*).

232   Despite differences in the content of the grains, the amount of phenolic acid in brown,

233   red, and black rice was found to be nearly the same. The different phenolic acids found in these

234   pigmented rice varieties include ferulic acid, p-cumaric, sinapic, ferulic, and hydroxybenzoic

235   acid. While ferulic, protocatechuic, and p-cumaric acids constituted the most prevalent common

236   cell wall-bound phenolic acids, sinapic, ferulic (28%) and vanillic acids constituted the most

237   significant soluble phenolic acids in black rice (*Blandino et al., 2022*). Black rice was found to

238   possess higher protocatechuic and vanillic acids than brown rice (*Zaupa et al., 2015*; *Shao et al.,*

239   *2018*). Using UV spectroscopy examination, the total phenolic content (TPC) of white, red and

240   black rice varieties was determined. White rice types had TPCs much lower than those of black

241   and red rice varieties (*Chen et al., 2022*). The genes involved in the biosynthesis of polyphenols

242   interrelate with each other and have particular functions in the control of the polyphenols levels

243   in rice grain (*Galland et al., 2014*). *OsCHS, OsCHI, OsF3H, OsF3'H, OsDFR*, and *OsANS* genes

244   from brown rice have the ability to change the yellow seed coat of *Arabidopsis thaliana* to

245   purplish. A yeast two-hybrid study revealed that, *OsCHS1, OsF3H, OsF3'H, OsDFR*, and

246   *OsANS1* interact with one another directly (*Shih et al., 2008*). The rice mutant *Rcrd* turns red

247 when *DFR* is introduced, demonstrating that Rd encodes the dihydroflavonol 4-reductase
248 (*Furukawa et al., 2007*).

249 *Polysaccharides*

250 Starch is the most abundant component which constitutes approximately 90% of rice grain. It is a
251 polyglucan of two polymers, amylase (linear) and amylopectin (highly branched) with α-1$\rightarrow$4-
252 linked linear glucans and α-1$\rightarrow$6-linked branches. Starch biosynthesis is a highly regulated
253 process that requires synchronized activities between various enzymes such as starch synthase
254 (SS), ADP-glucose pyro-phosphorylase (AGPase), starch branching enzyme (SBE) and de-
255 branching enzyme (DBE). Studies have reported that starch synthesis in all higher plants and
256 green algae and it is observed in higher plants, that the enzymes have undergone multi-sequential
257 changes throughout the process of evolution (*Qu et al., 2018*). Conserved mutations in the
258 enzymes- starch synthase SSIIIa and branching enzyme IIb (BEIIb) are helpful in breeding of
259 highly resistant starch with more health benefits (*Bao, 2019*). Several efforts have been made to
260 alter the expression and activities of starch biosynthetic enzyme by using various genetic as well
261 as molecular approaches. Transgenic rice plants have been beneficial for evaluating different
262 functions of the genes responsible as rice is easily transformed and a single DNA construct can
263 be used to produce a variety of transformed lines having different expression levels.

264 *Amylose and Amylopectin content modification by alteration of single gene function*

265 The modification of amylose content has been one among the most significant breeding
266 objectives because it affects gelatinization and cooking qualities. A lower amylose content rice is
267 usually selected as the milled grains are more appetizing than those with higher content
268 (*Denardin et al., 2012*). Amylose is synthesized by the granule-bound starch synthases (GBSS)
269 **(Figure 2)**. The amylose content in Japonica type rice (GBSSIb) in ss3a mutant was found
270 elevated when it replaced indica type rice (GBSSIa) (*Crofts et al., 2012*). The regulation of
271 *Os*GBSSI expression has been established in rice endosperm at the transcriptional as well as post
272 transcriptional levels and that accordingly differentiates these two varieties of rice-indica and
273 japonica in terms of amylose contents (*Liu et al., 2013*). The amylopectin content and its
274 structure may be directly altered by the isozymes involved in its biosynthesis and the extent of
275 such modification depends upon the isozyme's specificity. The change in amylopectin structure
276 for example alters the starch to be highly resistant for gelatinization because of more and long

277 double helices (*Miura et al., 2021*). There is also change in the X-ray diffraction patterns from
278 wild-type to mutant-type because the amylopectin has longer external chains. Similarly the
279 impact on amylopectin structure was seen when branching enzyme (BEIIb) activity in japonica
280 rice was down regulated by the RNAi approach (*Tsuiki et al., 2016*; *Zhang et al., 2022*). The
281 increase in amylopectin longer chains leads to an increase in the resistant starch content rather
282 than an increase in amylose content (*Tsuiki et al., 2016*).

283 *Modification of Amylose and Amylopectin Content by Alteration in Multiple Gene Functions*
284 Multiple gene functions leads to intense modifications in the starch biosynthesis and thus, the
285 phenotypes of these mutants exhibit variations. The changes are more extreme than predicted by
286 the single gene mutants and the plant usually doesn't survive. For example, the triple mutants
287 (ss1/ss2a/ss3a) of japonica rice type referred as null mutants becomes sterile which indicates the
288 functional properties of each isozyme and importance of the presence of at least one of these
289 (*SSI, SSIIa, and SSIIIa*) for starch biosynthesis in rice endosperm (*Fujita et al., 2011*). Other
290 indirect effects are also observed such as the ADP glucose concentrations were found enhanced
291 in the ss3a mutant as well as in ss3a/ss4b mutants when compared with wild type causing an
292 increase in the Amylose content (*Fujita et al., 2007*). Similarly, the *BEIIb* and *BEI* gene
293 inhibition results in the accumulation of amylose which leads to the different physico-chemical
294 properties in the rice endosperm providing highly resistant starch. DNA editing with
295 CRISPR/Cas9 twas found to be beneficial for specific editing of the *BEIIb* gene in rice for
296 improved starch structure (*Baysal et al., 2016*).

297 *Iron, Zinc and Micronutrients*
298 Rice grain has trace amounts of several vital micronutrients, including zinc (Zn), magnesium
299 (Mg), iron (Fe), copper (Cu), potassium (K), manganese (Mn) and calcium (Ca) (*Shao et al.,*
300 *2018*). However, the coloured rice possess greater amounts of Zn, Fe, and Mn when compared
301 with white rice (*Hurtada et al., 2018*; *Shao et al., 2018*). Brown rice can deliver up to 75% of the
302 optimal regular intake of zinc, copper, and iron while white rice only provides 37% (*Hashmi and*
303 *Tianlin, 2016*). Red rice from North-East India has been observed to contain highest content of
304 micronutrients (Al, K, Zn and Ca) than white rice (*Samyor et al., 2016*). A recent survey found
305 that two black rice variants from Korea, Heukjinjubyeo and Heukgwangbyeo possess greater
306 levels of Ca and K in comparison to a white variety (*Hiemori et al., 2009*). It has been also

307 observed that the ratio of micronutrients between coloured and non-coloured rice also differs.
308 The important nutrients in polished rice samples were found to be in the order of
309 K>Na>Mg>Zn>Fe>Mn>Cu>Cr, while in contrary to Brown rice variants, the order is:
310 K>Mg>Na>Mn>Zn>Fe>Cr>Cu (*Hashmi and Tianlin, 2016*). A survey was done in North East
311 India about the mineral content in black, red and white rice varieties and a complementary
312 investigation was conducted between mineral elements (Zn, Fe, Ca, Ni and Mn). The mineral
313 elements like Fe, Ca, Ni, Mn, Zn in various varieties of pigmented rice were present in highest
314 quantity than the white rice varieties (*Singh et al., 2022*). The growth conditions also affect the
315 Zn and Fe concentrations in the purple rice varieties. Wetland conditions were found to be more
316 advantageous than aerobic culture for producing purple rice with vivid coloration and greater
317 Zn(30 mg kg^{-1}) and Fe contents (15 mg kg^{-1}) (*Jaksomsak et al., 2021*).

318

319 *β-carotene*
320 Rice endosperm is not able to synthesize beta-carotene which acts as a precursor for vitamin A
321 synthesis leading to deficiency in vitamin A especially in the developing countries where rice is
322 the staple food and is the only way for fulfilling this nutritional requirement (*Das et al., 2020*).
323 Vitamin A or β-carotene is a most important micronutrient for proper vision and development in
324 humans and prevents many diseases such as night blindness, xerophthalmia and keratomalacia
325 (*Klemm et al., 2010*). As rice endosperm is lacking β-carotene so it was necessary to make rice
326 more nutritious by making it able to synthesize β-carotene (*Das et al., 2020*). In this context, an
327 experiment was designed by *Burkhardt et al. (1997)* in which they found, in rice endosperm,
328 geranyl geranyl diphosphate (GGPP), an important precursor for β-carotene biosynthesis making
329 rice endosperm capable of β-carotene synthesis. The Rice Taipei 309 (japonica variety) was
330 transformed with phytoene synthase from *Narcissus pseudonarcissus* by using micro projectile
331 bombardment leading to β-carotene synthesis (*Burkhardt et al., 1997*). In another study the β-
332 carotene content of Golden rice was enhanced ~23-fold by replacing the daffodil psy gene with
333 its homolog from maize and was named as Golden rice 2 (GR2) (*Mallikarjuna et al., 2021*)
334 which is now a good source of vitamin A and might become a part of many breeding programs in
335 Asia. Furthermore, β-carotene can be converted into the derivative astaxanthin which is keto-
336 carotenoid and red in color with high antioxidant activity but most higher plants are unable to
337 produce astaxanthin (*Ha et al., 2019*). Researchers genetically engineered *sPaCrtI* (phytoene

338   desaturase), *sZmPSY1* (phytoene synthase), s*HpBHY* (b-carotene hydroxylase), and *sCrBKT* (b-

339   carotene ketolase) genes to initiate the astaxanthin biosynthetic pathway to produce endospermic

340   astaxanthin in rice grains (*Zhu et al., 2018*). *Tian et al. (2019)* reported that bioengineering of

341   three chemically synthesized genes i.e., *tHMG1, ZmPSY1*, and *PaCRTI* in rice increased the

342   endospermic carotenoid biosynthesis through the mevalonate route. This engineering of

343   astaxanthin biosynthesis in rice endosperm converts Golden rice to aSTARice (*Zhu et al., 2018*).

344   These improved rice genotypes contain one more gene for β-carotene hydroxylase that produces

345   a red coloured Astaxanthin Rice (aSTARice). Therefore, biofortification of rice through

346   metabolic engineering could prove rice as a health promoting food and can be processed to

347   produce dietary supplements (*Zhu et al., 2018*).

348   *Folate*

349   Folates are a group of water-soluble B vitamins (B9), derived from most reduced folate form

350   known as tetrahydrofolate (THF) contains three building blocks—the pteridine, *p*-aminobenzoate

351   (*p*-ABA) and glutamate moieties (*Re'beille et al., 2006*). Living organisms use folates as C1

352   donors and acceptors and are mainly involved in the biosynthesis and metabolism of nucleotides,

353   amino acids and vitamin B5 (*Blancquaert* et al., *2010*). However, only plants and micro-

354   organisms can synthesize THF and its derivatives by de novo pathways. Therefore, humans are

355   dependent on food to meet their daily requirement of folates needed to regulate many

356   physiological and molecular processes (*Blancquaertet al., 2014*) for their survival. Many plants

357   like vegetables, pulses and fruits are loaded with folates but most staple crops such as rice which

358   is consumed by 1/2 of world's population; contain low levels of folate leads to folate deficiency

359   especially in developing countries (*Dong et al., 2014*). To eradicate folate deficiency worldwide

360   by biofortification of rice through metabolic engineering is a promising and cost-effective

361   approach. Moreover, the concept of enhancing folate content by over expressing the folate

362   biosynthesis genes/ metabolic engineering has been carried out in rice (*Strobbe and Van Der*

363   *Straeten, 2017*) and is an interesting target for improvement (*Re'beille et al., 2006*). The folate

364   biosynthesis pathway in plants is a multi-step process occurs in three different subcellular

365   compartments involves the conversion of chorismate (2 step process) by the action of ADC

366   synthase (ADCS) into p-aminobenzoate (pABA) in plastids. Genes involved in folate

367   biosynthesis such as *ADCS,GTPCHI, FPGS* and folate binding proteins (*FBP*) originated from

368   different organisms has been genetically engineered and over expressed in rice to produce higher

369   content of folates up to 100 fold (*Malik and Maqbool., 2020*) but further effective biofortification

370   strategy is needed. The lists of genes engineered in rice as a single or in combination by different

371   researchers to increase content of folates till now are mentioned in **Table 1.**

372   **Pigmentation in Rice**

373   Pigmented rice grains contain high levels of flavonoids, which are biosynthesized by two genes

374   (*CHS1* and *CHS2*) encoding chalcone synthetase located on chromosomes 11 and 7, respectively

375   (*Cheng et al., 2014; Lei et al., 2009*). Similarly, three flavone 3-hydroxylases, *F3H1, F3H2, and*

376   *F3H3*, contribute to proanthocyanidin production in red rice grains (*Park et al., 2016*) and the

377   two anthocyanin synthases are important for anthocyanins synthesis, ANS1 and ANS2 (*Shih et*

378   *al., 2008*).

379   *Red and White Pigmentation of Rice grain*

380   Rice grain colour was a major target during domestication and white rice was mostly selected,

381   while most wild type rice is red. The colour is determined by the functional activities of different

382   transcription factors. Two complementary genes, Rc and Rd (located on chromosomes 7 and 1,

383   respectively), encode a basic helix-loop-helix (bHLH) transcription factor and are responsible for

384   the red pericarp. RcRd genotypes produce red rice grain, while Rcrd genotypes produce brown

385   rice grain (*Furukawa et al., 2007*). The Rc gene is also involved in rice grain dormancy and

386   shattering. White variants have a loss-of-function mutation in the Rc allele (*Gross et al., 2010*).

387   *Purple Rice Pigmentation*

388   The purple-pericarp formation in rice is determined by the gene *Kala4/OsB2/Pb* which produces

389   anthocyanins (*Rahman et al., 2013*). Pigmentation variation is under polygenic control (*Ham et*

390   *al., 2015*). The Pl locus on chromosome 4 has three alleles (*Plw, Pli*, and *Plj)*, each responsible

391   for a different type of pigmentation. The wild type (*Plw*) produces anthocyanin in the aerial parts

392   of the rice plant. Pl locus possesses the two genes- *OSB*-1&2 encodes a helix loop helix

393   transcription factor (*Sakamoto et al., 2001*). Purple colour is characterized by the two dominant

394   genes Pb and Pp (*Ham et al., 2015*). A comparative genomics study in different rice lines by

395   *Lachagari et al. (2019)* revealed important allelic variants in genes related to flavonoid

396   synthesis, cytokinin glucosides, and betanidin degradation, and purple rice pigmentation.*Black*

397   *Rice Pigmentation*

398   The *Kala1, Kala3*, and *Kala4* loci located on chromosomes 1, 3, and 4 express black pericarp

399   traits in rice (*Maeda et al., 2014*). The pigments, mostly observed in the aleurone layers of black

400   rice, are a mixture of anthocyanins and range from black to dark purple. Variations in the Kala4

401   promoter sequence mostly result in black rice grain phenotypes. *Kala4* encodes a Helix Loop

402   Helix transcription factor which relates to *OSB2* gene responsible for the synthesis of

403   anthocyanins (*Sakulsingharoj et al., 2014*). Genetic studies, including QTL mapping and GWAS

404   analysis, have been used to understand the cause of rice pigmentation. Nine QTLs were reported

405   for flour pigmentation in an inbred line population (*Tan et al., 2001*), and four QTLs were

406   observed for red pigmentation (*Dong et al., 2008*). Twenty-one QTLs for variations in the

407   composition and content of proanthocyanidins and anthocyanins were identified in a study by *Xu*

408   *et al. (2017)*. Twenty-five marker trait associations for grain pigmentation were identified using

409   GWAS (*Shao et al., 2011*). GWAS has been more useful than QTL mapping in determining the

410   cause of rice pigmentation (*Korte and Farlow, 2013*). With the advance in GWAS, a total of 763

411   SNPs associated with pericarp pigmentation were reported by *Yang et al. (2018)* and some

412   specific SNPs were also identified associated with Rc (*Butardo et al., 2017*).

413   **Marker Trait Association Studies**

414   Quantitative trait locus (QTL) is a part of DNA that affects quantitative trait and QTL mapping is

415   a powerful and effective approach to analyse the chromosomal regions controlling quantitative

416   traits for the marker-assisted selection (MAS) strategy in rice (*Hu et al., 2021*; *Islam et al.,*

417   *2020*). Moreover, nutrient biofortification of rice by this method has been proved as a sustainable

418   strategy to overcome mineral deficiencies (*Majumder et al., 2019*). Mineral accumulation in

419   grain being a complex process and highly influenced by environmental factors has made

420   breeding and early-generation phenotypic-based selections of biofortified rice varieties slow and

421   less effective (*Sharma et al., 2020*). However, mapping major-effect QTLs by understanding

422   genetics of grain mineral elements at the molecular level would be helpful for the rapid

423   development of nutrient biofortification of rice varieties using marker-assisted breeding (MAB)

424   (*Swamy et al., 2018*). To date, different mapping populations derived from biparental inter- or

425   intra-subspecific and interspecific crosses have been used for identification of a large number of

426   QTLs in rice genome which are mainly associated with mineral contents (*Wang et al., 2020*) and

427   would be helpful in nutrient biofortification of rice varieties (*Hu et al., 2021*). In milled rice, 20

428   QTLs has been identified for P, K, Mg, Ca, Zn, Mn, and Cu contents (*Yu et al., 2015*) and in

429   another study 51 QTLs in brown rice and 61 QTLs in rice straw was identified for different

430   minerals (*Wang et al., 2020*) by using the RILs from the intra-subspecific cross Zhenshan

431   97/Miyang 46. *Garcia-Oliveira et al. (2009)* used introgression lines (ILs) from an interspecific

432   cross of cultivar "Teqing" and Yunnan wild rice (*O. rufipogon*) and reported 31 QTLs in brown

433   rice for P, K, Mg, Ca, Fe, Zn, Mn, and Cu contents. Some other researchers found 134 QTLS in

434   brown rice for 16 minerals content by using both RILs and backcross introgression lines (BILs)

435   from an inter-subspecific cross Lemont/Teqing (*Zhang et al., 2014*). *Descalsota-Empleo et al.*

436   *(2019)* used two sets of doubled haploid (DH) lines from two inter-subspecific crosses

437   IR64/IR69428 and BR29/IR75862 and reported 50 QTLs in milled rice for 13 minerals (P, K,

438   Na, Mg, and Ca). Furthermore, *Du et al. (2013)* showed influence of environmental factors in the

439   detection of QTL for grain mineral contents. In this study they selected brown rice grown in two

440   different ecological environments namely Lingshui and Hangzhou and mapped 23 and 9 QTLs

441   for seven mineral contents respectively and reported only two QTLs for the Mg content were

442   found in both the environments simultaneously. Several other reports based on QTLs for mineral

443   accumulation in rices have been detailed in **Table 2**.

444   **Conclusion and Future Prospectus**

445   Population growth and adverse global climatic changes negatively affect   our   food   and

446   nutritional securities which has resulted in hunger and malnutrition in our society. Rice

447   being a staple food for half of the world can be a source of energy for our generations

448   only if it is fortified with balanced nutrients. Pigmented rice as a food contain many bioactive

449   compounds that display significant potential concerning a range of beneficial health effects

450   like anti-cancerous, anti-allergic, anti-aging, anti-diabetic, and anti-obesity properties and

451   include many medicinal properties like treating ulcer, fracture, burns, skin lesions, and many

452   more. The extent of diseases faced today may be reduced significantly by simply replacing

453   white rice with pigmented rice in our day to day diet. The present limitations of low

454   productivity and palatability in pigmented rice can be solved by framing efficient breeding

455   strategies along with use of multiomics approaches. Development of highly palatable and high

456   yielding coloured rices will have a great impact in tackling various malnutrition concerns

457   observed in rice eating countries and this can have great implications in attaining nutritional

458   security.

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ADDITIONAL INFORMATION AND DECLARATIONS

Competing Interest

Authors declare no potential conflict of interest for this article.

Author contributions

- Jebi Sudan: conceived the idea, designed the experiment, performed the experiment, authored first draft of the article, analysed the data to contribute in preparing tables and figures and proof read the manuscript.
- Uneeb Urwat: performed the experiment, contributed in preparing first draft of the article, contributed in preparing tables and proof read the manuscript.
- Asmat Farooq: performed the experiment, contributed in preparing first draft, analysed the data to contribute in preparing tables and figures.
- Mohammad Maqbool Pakhtoon: performed the experiment, contributed in preparing first draft and analysed the data to contribute in preparing tables.
- Aaqif Zaffar: performed the experiment, contributed in preparing first draft of manuscript and proof read the manuscript.
- Zafir Ahmad Naik: performed the experiment, contributed in preparing first draft and contributed in preparing tables.
- Aneesa Batool: performed the experiment, contributed in preparing first draft of manuscript and contributed in preparing table.
- Saika Bashir: designed the experiment, contributed in preparing first draft of manuscript and contributed in preparing table.
- Madeeha Mansoor: designed the experiment, contributed in preparing first draft of manuscript and contributed in preparing table.

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499 • Sajad Majeed Zargar: conceived the idea, designed the experiment, contributed in the
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505
506

507 **Data Availability Statement**

508 Not applicable

509 **References**

510 **Ashraf H, Nazir Statistics N, Amir Wani IB, Naqash S, Husaini AM, Amjad Husaini CM,**
511   **Murtaza I, Nazir N, Wani AB. 2017.** Nutritional profiling of pigmented and scented rice
512   genotypes of Kashmir Himalayas. *Journal of Pharmacognosy and Phytochemistry***6**:910–
513   916.

514 **Bao J. 2019**. Rice starch. *In Rice* **55**:108. AACC International   DOI 10.1016/B978-0-12-
515   811508-4.00003-4

516 **Baysal C, Bortesi L, Zhu C, Farré G, Schillberg S, Christou P. 2016.** CRISPR/Cas9 activity
517   in the rice OsBEIIb gene does not induce off-target effects in the closely related paralog
518   OsBEIIa. *Molecular Breeding* **36**:1–11. DOI: 10.1007/s11032-016-0533-4.

519 **Bhat FM, Sommano SR, Riar CS, Seesuriyachan P, Chaiyaso T, Prom-U-thai C. 2020.**
520   Status of bioactive compounds from bran of pigmented traditional rice varieties and their
521   scope in production of medicinal food with nutraceutical importance. *Agronomy* **10**:1–15.
522   DOI: 10.3390/agronomy10111817.

523 **Biswas PS, Swamy BPM, Kader MA, Hossain MA, Boncodin R, Samia M, Hassan ML,**
524   **Wazuddin M, MacKenzie D, Reinke R. 2021**. Development and Field Evaluation of Near-
525   Isogenic Lines of GR2-EBRRI dhan29 Golden Rice. *Frontiers in Plant Science* **12.** DOI:

526   10.3389/fpls.2021.619739.

527 **Blancquaert D, De Steur H, Gellynck X, Van Der Straeten D. 2014.** Present and future of

528   folate biofortification of crop plants. *Journal of Experimental Botany* 65. DOI:

529   **10**.1093/jxb/ert483.

530 **Blancquaert D, Storozhenko S, Loizeau K, de Steur H, de Brouwer V, Viaene J, Ravanel S,**

531   **Rébeillé F, Lambert W, van der Straeten D. 2010.** Folates and folic acid: From

532   fundamental research toward sustainable health. *Critical Reviews in Plant Sciences* **29**.

533   DOI: 10.1080/07352680903436283.

534 **Blandino M, Bresciani A, Loscalzo M, Vanara F, Marti A. 2022.** Extruded snacks from

535   pigmented rice: Phenolic profile and physical properties. *Journal of Cereal Science* **103**.

536   DOI: 10.1016/j.jcs.2021.103347.

537 **Bollineni H, Gopala Krishnan S, Sundaram RM, Sudhakar D, Prabhu K V., Singh NK, Pal**

538   **M, Mishra S, Khurana JP, Singh AK. 2014.** Marker assisted biofortification of rice with

539   pro-vitamin A using transgenic Golden Rice® lines: Progress and prospects. *Indian Journal*

540   *of Genetics and Plant Breeding* **74**. DOI: 10.5958/0975-6906.2014.00901.8.

541 **Burkhardt PK, Beyer P, Wünn J, Klöti A, Armstrong GA, Schledz M, Von Lintig J,**

542   **Potrykus I. 1997**. Transgenic rice (Oryza sativa) endosperm expressing daffodil (Narcissus

543   pseudonarcissus) phytoene synthase accumulates phytoene, a key intermediate of

544   provitamin A biosynthesis. *Plant Journal* **11**. DOI: 10.1046/j.1365-313X.1997.11051071.x.

545 **Butardo VM, Anacleto R, Parween S, Samson I, de Guzman K, Alhambra CM, Misra G,**

546   **Sreenivasulu N. 2017.** Systems genetics identifies a novel regulatory domain of amylose

547   synthesis. *Plant Physiology* **173**. DOI: 10.1104/pp.16.01248.

548 **Chakuton K, Puangpronpitag D, Nakornriab M. 2012.** Phytochemical content and

549   antioxidant activity of colored and non-colored Thai rice cultivars. *Asian Journal of Plant*

550   *Sciences* 11. DOI: 10.3923/ajps.2012.285.293.

551 **Chen X, Yang Y, Yang X, Zhu G, Lu X, Jia F, Diao B, Yu S, Ali A, Zhang H, Xu P, Liao Y,**

552   **Sun C, Zhou H, Liu Y, Wang Y, Zhu J, Xiang Q, Wu X. 2022.** Investigation of flavonoid

553   components and their associated antioxidant capacity in different pigmented rice varieties.

554   *Food Research International* **161**. DOI: 10.1016/j.foodres.2022.111726.

555 **Cheng AX, Han XJ, Wu YF, Lou HX. 2014.** The function and catalysis of 2-oxoglutarate-

556   dependent oxygenases involved in plant flavonoid biosynthesis. *International journal of*

557   *molecular sciences*, **15(1),** 1080-1095.

558   **Crofts N, Abe K, Aihara S, Itoh R, Nakamura Y, Itoh K, Fujita N. 2012.** Lack of starch
559   synthase IIIa and high expression of granule-bound starch synthase I synergistically
560   increase the apparent amylose content in rice endosperm. *Plant Science* **193**. DOI:
561   10.1016/j.plantsci.2012.05.006.

562   **Dai S, Dunn ML, Park HS. 2010.** Piezoelectric constants for ZnO calculated using classical
563   polarizable core-shell potentials. *Nanotechnology* **21**. DOI: 10.1088/0957-
564   4484/21/44/445707.

565   **Das P, Adak S, Lahiri Majumder A. 2020.** Genetic Manipulation for Improved Nutritional
566   Quality in Rice. *Frontiers in Genetics* **11**. DOI: 10.3389/fgene.2020.00776.

567   **Denardin CC, Boufleur N, Reckziegel P, Silva LP da, Walter M. 2012.** Amylose content in
568   rice (Oryza sativa) affects performance, glycemic and lipidic metabolism in rats. *Ciência*
569   *Rural* **42**. DOI: 10.1590/s0103-84782012005000002.

570   **Deng GF, Xu XR, Zhang Y, Li D, Gan RY, Li H Bin. 2013.** Phenolic Compounds and
571   Bioactivities of Pigmented Rice. *Critical Reviews in Food Science and Nutrition* **53**:296–
572   306. DOI: 10.1080/10408398.2010.529624.

573   **Descalsota-Empleo GI, Amparado A, Inabangan-Asilo MA, Tesoro F, Stangoulis J, Reinke**
574   **R, Swamy BPM. 2019.** Genetic mapping of QTL for agronomic traits and grain mineral
575   elements in rice. *Crop Journal* **7**. DOI: 10.1016/j.cj.2019.03.002.

576   **Dong W, Cheng Z jun, Lei C lin, Wang X le, Wang J lin, Wang J, Wu F qing, Zhang X,**
577   **Guo X ping, Zhai H qu, Wan J min. 2014.** Overexpression of Folate Biosynthesis Genes
578   in Rice (Oryza sativa L.) and Evaluation of Their Impact on Seed Folate Content. *Plant*
579   *Foods for Human Nutrition* **69**. DOI: 10.1007/s11130-014-0450-9.

580   **Du J, Zeng D, Wang B, Qian Q, Zheng S, Ling HQ. 2013.** Environmental effects on mineral
581   accumulation in rice grains and identification of ecological specific QTLs. *Environmental*
582   *Geochemistry and Health* **35**. DOI: 10.1007/s10653-012-9473-z.

583   **Farré G, Sudhakar D, Naqvi S, Sandmann G, Christou P, Capell T, Zhu C. 2012.**
584   Transgenic rice grains expressing a heterologous ρ-hydroxyphenylpyruvate dioxygenase
585   shift tocopherol synthesis from the γ to the α isoform without increasing absolute tocopherol
586   levels. *Transgenic Research* **21**. DOI: 10.1007/s11248-012-9601-7.

587   **Fujita N, Satoh R, Hayashi A, Kodama M, Itoh R, Aihara S, Nakamura Y. 2011.** Starch

588   biosynthesis in rice endosperm requires the presence of either starch synthase i or IIIa.

589   *Journal of Experimental Botany* **62**. DOI: 10.1093/jxb/err125.

590   **Fujita N, Yoshida M, Kondo T, Saito K, Utsumi Y, Tokunaga T, Nishi A, Satoh H, Park**

591   **JH, Jane JL, Miyao A, Hirochika H, Nakamura Y. 2007.** Characterization of SSIIIa-

592   deficient mutants of rice: The function of SSIIIa and pleiotropic effects by SSIIIa deficiency

593   in the rice endosperm. *Plant Physiology* **144**. DOI: 10.1104/pp.107.102533.

594   **Furukawa T, Maekawa M, Oki T, Suda I, Iida S, Shimada H, Takamure I, Kadowaki KI.**

595   **2007.** The Rc and Rd genes are involved in proanthocyanidin synthesis in rice pericarp.

596   *Plant Journal* **49**. DOI: 10.1111/j.1365-313X.2006.02958.x.

597   **Galland C, Sangouard N, Piro N, Gisin N, Kippenberg TJ. 2014.** Heralded single-phonon

598   preparation, storage, and readout in cavity optomechanics. *Physical Review Letters* **112**.

599   DOI: 10.1103/PhysRevLett.112.143602.

600   **Garcia-Oliveira AL, Tan L, Fu Y, Sun C. 2009.** Genetic identification of quantitative trait loci

601   for contents of mineral nutrients in rice grain. *Journal of Integrative Plant Biology* **51**. DOI:

602   10.1111/j.1744-7909.2008.00730.x.

603   **Ghasemzadeh A, Karbalaii MT, Jaafar HZE, Rahmat A. 2018.** Phytochemical constituents,

604   antioxidant activity, and antiproliferative properties of black, red, and brown rice bran.

605   *Chemistry Central Journal* **12**. DOI: 10.1186/s13065-018-0382-9.

606   **Gross BL, Steffen FT, Olsen KM. 2010.** The molecular basis of white pericarps in African

607   domesticated rice: Novel mutations at the Rc gene. *Journal of Evolutionary Biology* **23**.

608   DOI: 10.1111/j.1420-9101.2010.02125.x.

609   **Gunaratne A, Wu K, Li D, Bentota A, Corke H, Cai YZ. 2013.** Antioxidant activity and

610   nutritional quality of traditional red-grained rice varieties containing proanthocyanidins.

611   *Food Chemistry* **138**. DOI: 10.1016/j.foodchem.2012.11.129.

612   **Ha SH, Kim JK, Jeong YS, You MK, Lim SH, Kim JK. 2019.** Stepwise pathway engineering

613   to the biosynthesis of zeaxanthin, astaxanthin and capsanthin in rice endosperm. *Metabolic*

614   *Engineering* **52**. DOI: 10.1016/j.ymben.2018.11.012.

615   **Ham T-H, Kwon SW, Ryu S-N, Koh H-J. 2015.** Correlation Analysis between Grain Color and

616   Cyanidin-3-glucoside Content of Rice Grain in Segregate Population. *Plant Breeding and*

617   *Biotechnology* **3**. DOI: 10.9787/pbb.2015.3.2.160.

618   **HAN L, DONG B cheng, YANG X ji, HUANG C bin, WANG X dong, WU X jun. 2009.**

619   Effect of Light on Flavonoids Biosynthesis in Red Rice Rdh. *Agricultural Sciences in China*
620   **8**. DOI: 10.1016/S1671-2927(08)60274-6.

621 **Hashmi MI, Tianlin JS. 2016.** Minerals contents of some indigenous rice varieties of Sabah
622   Malaysia. *International Journal of Agriculture, Forestry and Plantation* **2**:31-34

623 **Henriques JF, Serra D, Dinis TC, Almeida LM. 2020**. The anti-neuroinflammatory role of
624   anthocyanins and their metabolites for the prevention and treatment of brain disorders.
625   *International Journal of Molecular Sciences* **21(22)**:8653.

626 **Hiemori M, Koh E, Mitchell AE. 2009.** Influence of cooking on anthocyanins in black rice
627   (oryza sativa L. japonica var. SBR). *Journal of Agricultural and Food Chemistry* **57**. DOI:
628   10.1021/jf803153z.

629 **Hu BL, Li X, Wu T, Huang DR, Huang FL, Yin JH, Wu YS. 2021.** Identification and
630   Validation of QTLs for Macronutrient Contents in Brown and Milled Rice Using Two
631   Backcross Populations between Oryza sativa and O. rufipogon. *BioMed Research*
632   *International*. DOI: 10.1155/2021/5561734.

633 **Hurtada WA, Barrion ASA, Nguyen-Orca MFR. 2018.** Mineral content of dehulled and well-
634   milled pigmented and non-pigmented rice varieties in the Philippines. *International Food*
635   *Research Journal* **25**.

636 **Irakli MN, Samanidou VF, Katsantonis DN, Biliaderis CG, Papadoyannis IN. 2016.**
637   Phytochemical profiles and antioxidant capacity of pigmented and non-pigmented
638   genotypes of rice (Oryza sativa L.). *Cereal Research Communications* **44**. DOI:
639   10.1556/0806.43.2015.033.

640 **Islam MZ, Arifuzzaman M, Banik S, Hossain MA, Ferdous J, Khalequzzaman M,**
641   **Pittendrigh BR, Tomita M, Ali MP. 2020.** Mapping QTLs underpin nutrition components
642   in aromatic rice germplasm. *PLoS ONE* **15**. DOI: 10.1371/journal.pone.0234395.

643 **Jaksomsak P, Rerkasem B, Prom-U-Thai C. 2021**. Variation in nutritional quality of
644   pigmented rice varieties under different water regimes. *Plant Production Science* **24**. DOI:
645   10.1080/1343943X.2020.1819164.

646 **Jeng TL, Shih YJ, Ho PT, Lai CC, Lin YW, Wang CS, Sung JM. 2012.** γ-Oryzanol, tocol
647   and mineral compositions in different grain fractions of giant embryo rice mutants. *Journal*
648   *of the Science of Food and Agriculture* **92**. DOI: 10.1002/jsfa.4728.

649 **Juliano BO, Bechtel DB. 1985.** The grain and its gross composition. *Rice chemistry and*

650   *technology* **2:** 17-57.

651   **Kabera JN, Semana E, Mussa AR, He X. 2014.** Plant Secondary Metabolites: Biosynthesis,

652   Classification, Function and Pharmacological Properties. *Journal of Pharmacy and*

653   *Pharmacology* **2**: 45-85.

654   **Kaur P, Singh N, Pal P, Kaur A. 2018.** Variation in composition, protein and pasting

655   characteristics of different pigmented and non pigmented rice (Oryza sativa L.) grown in

656   Indian Himalayan region. *Journal of Food Science and Technology* **55**. DOI:

657   10.1007/s13197-018-3361-1.

658   **Kawakatsu T, Yamamoto MP, Hirose S, Yano M, Takaiwa F. 2008.** Characterization of a

659   new rice glutelin gene GluD-1 expressed in the starchy endosperm. *Journal of Experimental*

660   *Botany* **59**. DOI: 10.1093/jxb/ern265.

661   **Kim KH, Kabir E, Kabir S. 2015.** A review on the human health impact of airborne particulate

662   matter. *Environment International* **74**. DOI: 10.1016/j.envint.2014.10.005.

663   **Klemm RDW, West KP, Palmer AC, Johnson Q, Randall P, Ranum P, Northrop-Clewes C.**

664   **2010.** Vitamin a fortification of wheat flour: Considerations and current recommendations.

665   *Food and Nutrition Bulletin* **31**. DOI: 10.1177/15648265100311s105.

666   **Korte A, Farlow A. 2013.** The advantages and limitations of trait analysis with GWAS: A

667   review. *Plant Methods* **9.** DOI: 10.1186/1746-4811-9-29.

668   **Krishnan V, Singh A, Sharma S, Bollinedi H, Mabalirajan U, Kota S, Raja S, Singh AK,**

669   **Praveen S. 2020**. Nutrient dense pigmented rice: A diet for healthier people. Published by

670   Division of Biochemistry ICAR-Indian Agricultural Research Institute New Delhi.

671   **Lachagari VBR, Gupta R, Lekkala SP, Mahadevan L, Kuriakose B, Chakravartty N,**

672   **Mohan Katta AVSK, Santhosh S, Reddy AR, Thomas G. 2019.** Whole genome

673   sequencing and comparative genomic analysis reveal allelic variations unique to a purple

674   colored rice landrace (Oryza sativa ssp. Indica cv. Purpleputtu). *Frontiers in Plant Science*

675   **10**. DOI: 10.3389/fpls.2019.00513.

676   **Liu DR, Huang WX, Cai XL. 2013.** Oligomerization of rice granule-bound starch synthase 1

677   modulates its activity regulation. *Plant Science* **210**. DOI: 10.1016/j.plantsci.2013.05.019.

678   **Mackon E, Jeazet Dongho Epse Mackon GC, Ma Y, Kashif MH, Ali N, Usman B, Liu P.**

679   **2021.** Recent insights into anthocyanin pigmentation, synthesis, trafficking, and regulatory

680   mechanisms in rice (Oryza sativa l.) caryopsis. *Biomolecules* **11**. DOI:

681   10.3390/biom11030394.

682   **Maeda H, Yamaguchi T, Omoteno M, Takarada T, Fujita K, Murata K, Iyama Y, Kojima**
683   **Y, Morikawa M, Ozaki H, Mukaino N, Kidani Y, Ebitani T. 2014.** Genetic dissection of
684   black grain rice by the development of a near isogenic line. *Breeding Science* **64**. DOI:
685   10.1270/jsbbs.64.134.

686   **Majumder S, Datta K, Datta SK. 2019.** Rice biofortification: High iron, zinc, and Vitamin-A
687   to fight against "hidden hunger." *Agronomy* **9**. DOI: 10.3390/agronomy9120803.

688   **Malik KA, Maqbool A. 2020.** Transgenic Crops for Biofortification. *Frontiers in Sustainable*
689   *Food Systems* **4**. DOI: 10.3389/fsufs.2020.571402.

690   **Mallikarjuna Swamy BP, Marundan S, Samia M, Ordonio RL, Rebong DB, Miranda R,**
691   **Alibuyog A, Rebong AT, Tabil MA, Suralta RR, Alfonso AA, Biswas PS, Kader MA,**
692   **Reinke RF, Boncodin R, MacKenzie DJ. 2021.** Development and characterization of
693   GR2E Golden rice introgression lines. *Scientific Reports* **11**. DOI: 10.1038/s41598-021-
694   82001-0.

695   **Mbanjo EGN, Kretzschmar T, Jones H, Ereful N, Blanchard C, Boyd LA, Sreenivasulu N.**
696   **2020.** The Genetic Basis and Nutritional Benefits of Pigmented Rice Grain. *Frontiers in*
697   *Genetics* **11**. DOI: 10.3389/fgene.2020.00229.

698   **Melini V, Acquistucci R. 2017.** Health-promoting compounds in pigmented thai and wild rice.
699   *Foods* **6**. DOI: 10.3390/foods6010009.

700   **Miura S, Koyama N, Crofts N, Hosaka Y, Abe M, Fujita N. 2021.** Generation and Starch
701   Characterization of Non-Transgenic BEI and BEIIb Double Mutant Rice (Oryza sativa)
702   with Ultra-High Level of Resistant Starch. *Rice* **14**. DOI: 10.1186/s12284-020-00441-0.

703   **Ostlund RE. 2002.** Phytosterols in human nutrition. *Annual Review of Nutrition* **22**. DOI:
704   10.1146/annurev.nutr.22.020702.075220.

705   **Panche AN, Diwan AD, Chandra SR. 2016.** Flavonoids: An overview. *Journal of Nutritional*
706   *Science* **5**. DOI: 10.1017/jns.2016.41.

707   **Park S, Choi MJ, Lee JY, Kim JK, Ha SH, Lim SH. 2016.** Molecular and biochemical
708   analysis of two rice flavonoid 3'-hydroxylase to evaluate their roles in flavonoid
709   biosynthesis in rice grain. *International Journal of Molecular Sciences* **17(9):**1549.

710

711   **Pereira-Caro G, Watanabe S, Crozier A, Fujimura T, Yokota T, Ashihara H. 2013.**

712   Phytochemical profile of a Japanese black-purple rice. *Food Chemistry* **141**. DOI:
713   10.1016/j.foodchem.2013.05.100.

714 **Perera CO, Yen GM. 2007.** Functional properties of carotenoids in human health. *International*
715   *Journal of Food Properties* **10**. DOI: 10.1080/10942910601045271.

716 **Petroni K, Landoni M, Tomay F, Calvenzani V, Simonelli C, Cormegna M. 2017.** Proximate
717   Composition, Polyphenol Content and Anti-inflammatory Properties of White and
718   Pigmented Italian Rice Varieties. *Universal Journal of Agricultural Research* **5**. DOI:
719   10.13189/ujar.2017.050509.

720 **Qu J, Xu S, Zhang Z, Chen G, Zhong Y, Liu L, Zhang R, Xue J, Guo D. 2018.** Evolutionary,
721   structural and expression analysis of core genes involved in starch synthesis. *Scientific*
722   *Reports* **8**. DOI: 10.1038/s41598-018-30411-y.

723 **Rahman MM, Lee KE, Lee ES, Matin MN, Lee DS, Yun JS, Kim JB, Kang SG. 2013.** The
724   genetic constitutions of complementary genes Pp and Pb determine the purple color
725   variation in pericarps with cyanidin-3-O-glucoside depositions in black rice. *Journal of*
726   *Plant Biology* **56**. DOI: 10.1007/s12374-012-0043-9.

727 **Rani S, Pooja K, Pal GK. 2018.** Exploration of rice protein hydrolysates and peptides with
728   special reference to antioxidant potential: Computational derived approaches for bio-
729   activity determination. *Trends in Food Science and Technology* **80**. DOI:
730   10.1016/j.tifs.2018.07.013.

731 **Rébeillé F, Ravanel S, Jabrin S, Douce R, Storozhenko S, Van Der Straeten D. 2006.** Folates
732   in plants: Biosynthesis, distribution, and enhancement. *Physiologia Plantarum* **126**. DOI:
733   10.1111/j.1399-3054.2006.00587.x.

734 **Rengasamy KR, Khan H, Gowrishankar S, Lagoa RJ, Mahomoodally FM, Khan Z,**
735   **Suroowan S, Tewari D, Zengin G, Hassan ST, Pandian SK. 2019.** The role of flavonoids
736   in autoimmune diseases: Therapeutic updates. *Pharmacology & therapeutics* **194**: 107-131.

737 **Sakamoto W, Ohmori T, Kageyama K, Miyazaki C, Saito A, Murata M, Noda K, Maekawa**
738   **M. 2001.** The Purple leaf (Pl) locus of rice: The Plw allele has a complex organization and
739   includes two genes encoding basic helix-loop-helix proteins involved in anthocyanin
740   biosynthesis. *Plant and Cell Physiology* **42**. DOI: 10.1093/pcp/pce128.

741 **Sakulsingharoj C, Inta P, Sukkasem R, Pongjaroenkit S, Chowpongpang S, Sangtong V.**
742   **2014**. Overexpression of OSB2 gene in transgenic rice up-regulated expression of structural

743   genes in anthocyanin biosynthesis pathway. *Genomics and Genetics* **7(3)**:173-
744   182.https://doi.org/10.14456/tjg.2014.24

745 **Samanta Amalesh, Das Gouranga DSK. 2011.** Roles of flavonoids in Plants. *Int J Pharm Sci*
746   *Tech* **100(6)**:12-35.

747 **Samyor D, Das AB, Deka SC. 2017.** Pigmented rice a potential source of bioactive compounds:
748   a review. *International Journal of Food Science and Technology* **52**. DOI:
749   10.1111/ijfs.13378.

750 **Samyor D, Deka SC, Das AB. 2016.** Phytochemical and antioxidant profile of pigmented and
751   non-pigmented rice cultivars of Arunachal Pradesh, India. *International Journal of Food*
752   *Properties* **19**. DOI: 10.1080/10942912.2015.1055761.

753 **Sati R, Singh S. 2019.** Pigmented rice: A potential ingredient for extruded products review
754   paper. *~ 700 ~ Journal of Pharmacognosy and Phytochemistry* **8**: 87-110/

755 **Schaefer EJ. 2002.** Lipoproteins, nutrition, and heart disease. *American Journal of Clinical*
756   *Nutrition* **75**. DOI: 10.1093/ajcn/75.2.191.

757 **Shao Y, Jin L, Zhang G, Lu Y, Shen Y, Bao J. 2011**. Association mapping of grain color,
758   phenolic content, flavonoid content and antioxidant capacity in dehulled rice. *Theoretical*
759   *and Applied Genetics* **122**. DOI: 10.1007/s00122-010-1505-4.

760 **Sharma V, Kumar Saini D, Kumar A, Kesh H, Kaushik P. 2020.** Breeding for
761   Biofortification Traits in Rice: Means to Eradicate Hidden Hunger. In: *Agronomy - Climate*
762   *Change and Food Security*. DOI: 10.5772/intechopen.91144.

763 **Shih CH, Chu H, Tang LK, Sakamoto W, Maekawa M, Chu IK, Wang M, Lo C. 2008.**
764   Functional characterization of key structural genes in rice flavonoid biosynthesis. *Planta*
765   **228**. DOI: 10.1007/s00425-008-0806-1.

766 **Singh SP, Vanlalsanga, Mehta SK, Singh YT. 2022.** New insight into the pigmented rice of
767   northeast India revealed high antioxidant and mineral compositions for better human health.
768   *Heliyon* **8**. DOI: 10.1016/j.heliyon.2022.e10464.

769 **Sornkhwan T, Chinvongamorn C, Sansenya S. 2022.** Effect of Bio-Liquid Fertilizer from
770   Saltpan Residual on the Gamma-Oryzanol Content of Germinated Brown Rice (Thai
771   Pigmented Rice). *Progress in Applied Science and Technology* **12(2)**: 6-13.

772 **Strobbe S, Van Der Straeten D. 2017.** Folate biofortification in food crops. *Current Opinion in*
773   *Biotechnology* **44.** DOI: 10.1016/j.copbio.2016.12.003.

774 **Swamy BPM, Descalsota GIL, Nha CT, Amparado A, Inabangan-Asilo MA, Manito C,**
775   **Tesoro F, Reinke R. 2018.** Identification of genomic regions associated with agronomic
776   and biofortification traits in DH populations of rice. *PLoS ONE* **13**. DOI:
777   10.1371/journal.pone.0201756.

778 **Tan YF, Sun M, Xing YZ, Hua JP, Sun XL, Zhang QF, Corke H. 2001.** Mapping
779   quantitative trait loci for milling quality, protein content and color characteristics of rice
780   using a recombinant inbred line population derived from an elite rice hybrid. *Theoretical*
781   *and Applied Genetics* **103**. DOI: 10.1007/s001220100665.

782 **Tian YS, Wang B, Peng RH, Xu J, Li T, Fu XY, Xiong AS, Gao JJ, Yao QH. 2019.**
783   Enhancing carotenoid biosynthesis in rice endosperm by metabolic engineering. *Plant*
784   *Biotechnology Journal* **17**. DOI: 10.1111/pbi.13059.

785 **Tsuiki K, Fujisawa H, Itoh A, Sato M, Fujita N. 2016.** Alterations of starch structure lead to
786   increased resistant starch of steamed rice: Identification of high resistant starch rice lines.
787   *Journal of Cereal Science* **68**. DOI: 10.1016/j.jcs.2016.01.002.

788 **Ustunol Z. 2014.** Overview of Food Proteins. In: *Applied Food Protein Chemistry*. DOI:
789   10.1002/9781118860588.ch2.

790 **Wakasa K, Hasegawa H, Nemoto H, Matsuda F, Miyazawa H, Tozawa Y, Morino K,**
791   **Komatsu A, Yamada T, Terakawa T, Miyagawa H. 2006.** High-level tryptophan
792   accumulation in seeds of transgenic rice and its limited effects on agronomic traits and seed
793   metabolite profile. *Journal of Experimental Botany* **57**. DOI: 10.1093/jxb/erl068.

794 **Wang X, Song YE, Li JY. 2013.** High expression of tocochromanol biosynthesis genes
795   increases the vitamin e level in a new line of giant embryo rice. *Journal of Agricultural and*
796   *Food Chemistry* **61**. DOI: 10.1021/jf401325e.

797 **Wang CC, Tang Z, Zhuang JY, Tang Z, Huang XY, Zhao FJ. 2020.** Genetic mapping of
798   ionomic quantitative trait loci in rice grain and straw reveals OsMOT1;1 as the putative
799   causal gene for a molybdenum QTL qMo8. *Molecular Genetics and Genomics* **295**. DOI:
800   10.1007/s00438-019-01632-1.

801 **Wong HW, Liu Q, Sun SSM. 2015.** Biofortification of rice with lysine using endogenous
802   histones. *Plant Molecular Biology* **87**. DOI: 10.1007/s11103-014-0272-z.

803 **Wongsa P. 2021.** Phenolic Compounds and Potential Health Benefits of Pigmented Rice. In:
804   *Recent Advances in Rice Research*. DOI: 10.5772/intechopen.93876.

805 **Wu XR, Chen ZH, Folk WR. 2003**. Enrichment of cereal protein lysine content by altered
806   tRNAlys coding during protein synthesis. *Plant Biotechnology Journal* **1**. DOI:
807   10.1046/j.1467-7652.2003.00017.x.

808 **Xu TY, Sun J, Chang HL, Zheng HL, Wang JG, Liu HL, Yang LM, Zhao HW, Zou DT.**
809   **2017**. QTL mapping for anthocyanin and proanthocyanidin content in red rice. *Euphytica*
810   **213**. DOI: 10.1007/s10681-017-2035-9.

811 **Yang Y, Liu Y, Lv X, Ai J, Li Y. 2022**. Anthropomorphism and customers' willingness to use
812   artificial intelligence service agents. *Journal of Hospitality Marketing and Management* **31**.
813   DOI: 10.1080/19368623.2021.1926037.

814 **Yang X, Xia X, Zeng Y, Nong B, Zhang Z, Wu Y, Xiong F, Zhang Y, Liang H, Deng G, Li**
815   **D. 2018**. Identification of candidate genes for gelatinization temperature, gel consistency
816   and pericarp color by GWAS in rice based on SLAF-sequencing. *PLoS ONE* **13**. DOI:
817   10.1371/journal.pone.0196690.

818 **Yu YH, Shao YF, Liu J, Fan YY, Sun CX, Cao ZY, Zhuang JY. 2015.** Mapping of
819   Quantitative Trait Loci for Contents of Macro- and Microelements in Milled Rice (Oryza
820   sativa L.). *Journal of Agricultural and Food Chemistry* **63**. DOI: 10.1021/acs.jafc.5b02882.

821 **Zaupa M, Calani L, Del Rio D, Brighenti F, Pellegrini N. 2015.** Characterization of total
822   antioxidant capacity and (poly)phenolic compounds of differently pigmented rice varieties
823   and their changes during domestic cooking. *Food Chemistry* **187**. DOI:
824   10.1016/j.foodchem.2015.04.055.

825 **Zhang Z, Hu Y, Zhao J, Zhang Y, Ying Y, Xu F, Bao J. 2022.** The role of different Wx and
826   BEIIb allele combinations on fine structures and functional properties of indica rice
827   starches. *Carbohydrate Polymers* **278.** DOI: 10.1016/j.carbpol.2021.118972.

828 **Zhang M, Pinson SRM, Tarpley L, Huang XY, Lahner B, Yakubova E, Baxter I, Guerinot**
829   **M Lou, Salt DE. 2014.** Mapping and validation of quantitative trait loci associated with
830   concentrations of 16 elements in unmilled rice grain. *Theoretical and Applied Genetics* **127**.
831   DOI: 10.1007/s00122-013-2207-5.

832 **Zhu Q, Zeng D, Yu S, Cui C, Li J, Li H, Chen J, Zhang R, Zhao X, Chen L, Liu YG. 2018.**
833   From Golden Rice to aSTARice: Bioengineering Astaxanthin Biosynthesis in Rice
834   Endosperm. *Molecular Plant* **11**. DOI: 10.1016/j.molp.2018.09.007.

835 **Zubair M, Anwar F, Ashraf M, Uddin MK. 2012.** Characterization of high-value bioactives in

836   some selected varieties of Pakistani rice (Oryza sativa L.). *International journal of*
837   *molecular sciences* **13(4)**:4608-4622.

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842   **<u>Figure legends</u>**

843   **Figure 1.** The importance of pigmented rice in India with respect to the (a) varieties cultivated in
844   different parts; (b) elemental content in different coloured-rice and (c) different nutraceutical
845   benefits of pigmented rice.

846   **Figure 2.** Genes Regulating Starch Biosynthesis in the endosperm through various
847   transcriptional factors, different abbreviations indicates amylose content (AC), eating & cooking
848   quality (ECQ), nutritional quality (NQ) and appearance quality(AQ), granule-bound starch
849   synthase (GBSS), soluble starch synthase (SS) and starch branching enzymes (SBE**).**

850   **<u>Table legends</u>**

851   **Table 1.** Genes engineered in rice seed for different nutrients and metabolites

852   **Table 2.** QTL studies showing different markers trait associations for nutrient enhancement in
853   rice

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Table 1(on next page)

Table 1

Genes engineered in rice seed for different nutrients and metabolites

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2   **Table 1.** Genes engineered in rice seed for different nutrients and metabolites

Nutrient/ Metabolite	Gene Origin	Engineering Approach	Genes Transformed	Expression Details/ Fold change in expression	References
Folate	*Arabidopsis thaliana*	Biosynthesis Single-gene	ADCS	(1/6)-fold upregulated	*Strobbe and Van DerStraeten., 2017*
	Arabidopsis thaliana	Biosynthesis Single	GTPCHI	6.1-fold	
	Arabidopsis thaliana	Biosynthesis Single	HPPK/DHPS	1.4-fold	
	Triticum aestivum	Biosynthesis Single	DHFS	1.27-fold	
	Arabidopsis thaliana	Biosynthesis Multi-gene	GTPCHI + other biosynthesis genes	6.1-fold	
	Arabidopsis thaliana	Biosynthesis Multi-gene	GTPCHI + ADCS	100-fold	
	Arabidopsis thaliana	Polyglutamylation	FPGS	1.45-fold	
	Oryza sativa	Polyglutamylation	FPGS	4.7-fold	
	Arabidopsis thaliana	Polyglutamylation	GTPCHI + ADCS + FPGS	100-fold	
	Bos taurus	Folate binding proteins	FBP	6.2-fold	
	Rattus norvegicus	Folate binding proteins	GNMT	8.8-fold	
	Arabidopsis thaliana (G + A) Bostaurus (FBP)	Folate binding proteins	GTPCHI + ADCS + FBP	150-fold	
Lysine	*Arabidopsis thaliana*	Manipulation of lysine content	DHDPS	Feedback inhibition	*Das et al. 2020*

Carotenoids	*Zea mays*	Agrobacterium mediated DNA transfer	*OsLCYB*	1.9-fold upregulated	*Tian et al. 2019*
Carotenoids	*Pantoea ananatis*	Agrobacterium mediated DNA transfer	*OsBCH2*	1.1-fold upregulated	*Tian et al. 2019*
	Saccharomyces cerevisiae	Agrobacterium mediated DNA transfer	*OsPDS*	1.6-fold upregulated	*Tian et al. 2019*
Vitamin A	*Narcissus pseudonarcissus*	Transformation by microprojectile bombardment	*pCPsyH*	Expression observed in only the transformed plants.	*Burkhardt et al. 1997*
Vitamin E	*Oryza sativa*	Single point mutation	Giant embryo gene (ge)	2.2-fold upregulated	*Wang et al. 2013*
	Arabidopsis thaliana	Transformation by particle bombardment	*PDS1*	upregulated	*Farre et al. 2012*
Starch	*Solanum tuberosum*	Agrobacterium-mediated transformation	StGWD1	9-fold higher 6-phospho (6-P) monoesters and double amounts of 3-phospho (3-P) monoesters.	*Chen et al. 2017*
	Zea mays	Agrobacterium-mediated transformation	OsSUS1-6	Upregulatedstarch accumulation for improved grain filling.	*Fan et al. 2019*

	Oryza sativa	Map-based cloning-Mutation of T-DNA(gamma-radiated hybrid-rice)	SSIII and Waxy (Wx)	High in resistant starch (RS)	*Zhou et al. 2016*
	Arabidopsis thaliana	RNA interference approach	Starch Excess 4 (SEX4)	improved bioethanol yield, with a 50% increase in ethanol production	*Huang et al. 2020*
	Oryza sativa	RNA interference approach (antisenseWx gene)	Wx	amylose content in transgenic caryopsis was downregulated	*Chen et al. 2006; Khandagale et al. 2018*
	Thermoan aerobacter ethanolicus	Agrobacterium tumefaciens mediated transfer	APU (amylopullulanase)	Reduction of amylose, altered starch properties	*Chiang et al. 2005*
Nitrogen	*Arabidopsis thaliana*	Constitutive overexpression	MYB12, MYC, WD40	Upregulated	*Lee et al. 2016*
Phosphorus	*Arabidopsis thaliana*	Constitutive overexpression	OsMYB3R-2	Upregulated	*Huang et al. 2018*

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Table 2(on next page)

Table 2

QTL studies showing different markers trait associations for nutrient enhancement in rice

1   **Table 2.** QTL studies showing different markers trait associations for nutrient enhancement in rice

Grain Traits	QTL	Trait marker	References
Zinc (Zn)	*qZN-5, qZN-7, qSZn2, qSZn12, qZn7, qZn3.1, qZn7.1, qZn7.2, qZn7.3, qZn12.1, qZn4, qZn6, qZn1-1, qZn12-1, qZn5-1, qZn8-1, qZn12-1, qZn2.1, qZn2.1, qZn3.1, qZn6.1, qZn6.2, qZn8.1, qZn11.1, qZn12.1, qZn12.2, qZn2.2, qZn8.3, qZn12.3, qZn3.1, qZn7, qZn8.3, qZn3-1, qZn1.1, qZn5.1, qZn9.1, qZn12.1, qZn1.1, qZn6.1, qZn6.2, qZn2-1, qZn2-2, qZn5, qZn10, qZn2.1, qZn3.1, qZn5.1, qZn5.2, qZn7.1, qZn9.1, qZn11.1, qZn 1.1, qZn2.1, qZn3.1, qZn3.2, qZn5.1, qZn6.1, qZn8.1, qZn8.2, qZn9.1, qZn12.1, QTL.Zn.4, QTL.Zn.5*	RMID2009463, RM2147095, RM2785595, RM6047367, RMID6006214, RM8832534, RMID11000778,RM12985052, RM13057679,RM34-RM237, RM7-RM517, RZ398-RM204 RM501-OsZip2, RM152, RM25-R1629, RM235-RM17, RM260-RM7102, RM551, RM413	*Das et al. 2020; Islam et al. 2020; Thangadurai et al. 2020; Swamy et al. 2018*
Iron (Fe)	*qFE-1, qFE-9, qGFe4, qSFe1, qSFe12, qFe1, qFe3, qFe6, qFe2-1, qFe9-1, qFe4.1, qFe3.3, qFe7.3, Fe8.1, qFe12.2, qFe3-1, qFe9.1, qFe12.1, qFe1.1, qFe1.2, qFe6.1, qFe6.2, qFe5, qK6.1, qFe2.2, qFe3.1, qFe4.1., qFe6.1, qFe8.1, qFe11.2, qFe11.3, qFe12.1,*	RM4743351, RM574-RM122, RM234-RM248, RM137-RM325A, RZ536-TEL3, RM270-RM17, RM260-RM7102, RM17-RM260, RM452, RM215	*Das et al. 2020; Islam et al. 2020; Thangadurai et al. 2020; Swamy et al. 2018*
Manganese (Mn)	*qMn1-1, qMn2-1, qMn3-1, qMn10-1, qMn2.1, qMn2.1, qMn7.1, qMn1.1, qMn1.2, qMn3.1, qMn3.2, qMn4.1*	RM243-RM312, RM6367, RM227-R1925, RM214, RMID2009186, RMID2009463, RM7592793	*Das et al. 2020; Mahender et al. 2016; Swamy et al. 2018*
Calcium	*qCa1-1, qCa4-1, qCa5-1, qCa9-1, qCa10-1, qCa11-1, qCa12-1, qCa1.1, qCa1.1, qCa2.1, qCa2.1, qCa3.1, qCa3.2,*	RM403585, RMID1013855, RMID2009186, RM2131264, RM2499734, RM2733626, RM598, RM5626-RM16, RM200-RM227,	*Das et al. 2020; Mahender et al. 2016; Swamy et al. 2018*

Magnesium (Mg)	*qMg1-1, qMg3-1, qMg5-1, qMg9-1, qMg12-1, qMg3.1, qMg3.2, qMg5.1, qMg8.1, qMg9.1, qMg1.1, qMg7.1, qMg8.1, qMg11.1*	RM2499734, RM3460782, RM5522491, RM8892951, RM9886119,OSR 21, RM467, RM332,	*Das et al. 2020; Mahender et al. 2016; Swamy et al. 2018*
Phosphorus (P)	*qP1-1, qP3-1, qP8-1, qP9-1, qP12-1, qP1.1, qP2.1, qP2.2, qP5.1, qP6.1, qP11.1, qP11.2*	RM,119519, RMID2009186, RM2181296, RM5430212, RMID6009257, RM3411, RM495, RM212, RM70-RM172, RM201,	*Das et al. 202; Mahender et al. 2016; Swamy et al. 2018*
Potassium (K)	*qK1-1 qK.1, qK4-1, qK8-1, qK9-1, qK2.1, qK4.1, qK4.2, qK5.1, qK9.1, qK3.1, qK3.2, qK3.3, qK4.1, qK5.1,*	RM2094246, RM4285667, RM4668476, RM5430212, RM9858839, RM3572, RM5501,	*Das et al. 2020; Mahender et al. 2016; Swamy et al. 2018*
Boron (B)	*qB2.1, qB3.1, qB4.1, qB4.2, qB10.1*	RMid2009186, RM2645329, RM4314701,	*Das et al. 2020; Swamy et al. 2018*
Cobalt (Co)	*qCo1.1, qCo3.1, qCo4.1, qCo12.1, qCo7.1, qCo10.1*	RM827062, RM2785595,RM4572241, RM12958034	*Das et al. 2020; Swamy et al. 2018*
Copper (Cu)	*qCu3.1, qCu4.1, qCu4.2, qCu1.1, qCu1.2, qCu2.1, qCu6.1, qCu8.1*	RM3330180, RM4314701, RM4761773	*Das et al. 2020; Swamy et al. 2018*
Molybdenum (Mo)	*qMo1.1, qMo1.2, qMo1.3, qMo2.1, qMo11.1, qMo12.1, qMo12.1, qMo12.1*	RM854218, RMID1014853, RM1191519, RM1725183, RMID11006537, RM13030749, RM13044018	*Das et al. 2020; Swamy et al. 2018*
Sodium (Na)	*qNa1.1, qNa1.2, qNa7.1, qNa7.2, qNa10.1, qNa3.1, qNa11.1, qNa11.2*	RM267954, RM784044,RMID7003294,RM7 962882, RM10635878	*Das et al. 2020; Swamy et al. 2018*

Phytic acid (PA)	*qPA.12*	RM247-RM179	*Thangadurai et al. 2020*
Grain protein content (GPC)	*qPC1, qPC2, qPC3, qPC6.1, qPC6.2, qPC8, qPC12.1, qPC1.1, qPC11.1, and qPC11.2, qPC-3, qPC-4, qPC-5, qPC-6 and qPC-10, qPr1 and qPr7, qPro-8, qPro-9 and qPro-10, qGPC1.1, qSGPC2.1 and qSGPC7.1,QTL.pro.1*	RM493-RM562, 1008-RM575, RM472-RM104, RM5619-RM1211, RM12532-RM555, RM251-RM282, RM190-RZ516, RM190-RZ516, RM270-C751, R1245-RM234, RM445-RM418, RM184-RM3229B, RM24934-RM25128, 1027-RM287, RM287-RM26755, RM5	*Thangadurai et al. 2020; Islam et al. 2020; Mahender et al. 2016*
Amino acid content (AAC)	*qAa1, qAa7, qAA.8, qAA.4, qAA.3, qAA.2, qAA.1, qAa9, qAA.10*	RM493-RM562, RM472-RM104, RM324-RM301, RM322-RM521, RM520-RM468, RM348-RM131, RM125-RM214, RM137-RM556, RM447-RM458, RM328-RM107, RM467-RM271	*Thangadurai et al. 2020; Mahender et al. 2016*
Amylose (amy)	*QTL.amy.6, QTL.amy.7, QTL.amy.8, QTL.amy.11*	RM190, RM125, RM284, RM144	*Islam et al. 2020*

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Figure 1

Figure 1

The importance of pigmented rice in India with respect to the (a) varieties cultivated in different parts; (b) elemental content in different coloured-rice and (c) different nutraceutical benefits of pigmented rice.
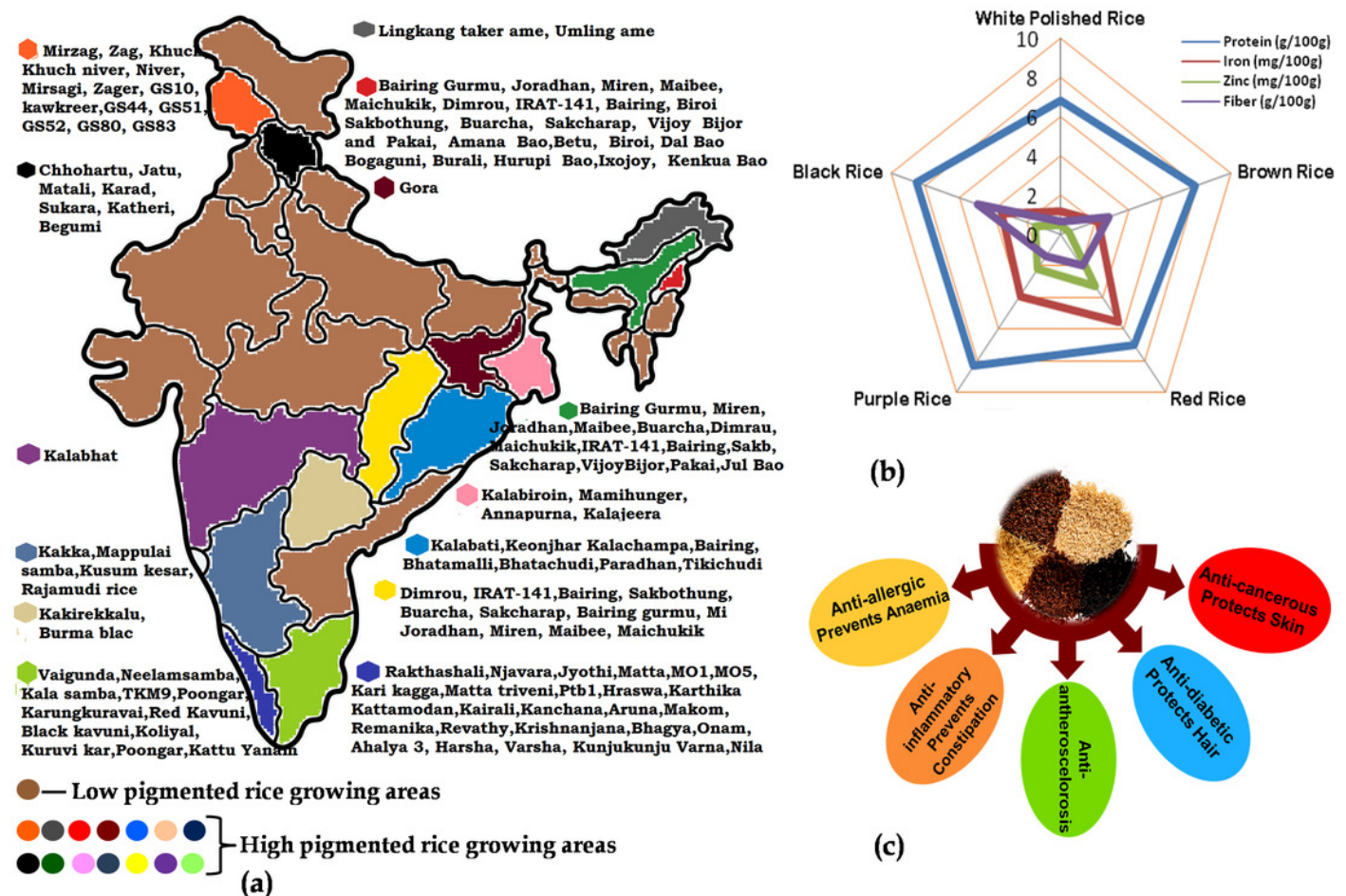
Figure 2

Figure 2

Genes Regulating Starch Biosynthesis in the endosperm through various transcriptional factors where, different abbreviations indicates amylose content (AC), eating & cooking quality (ECQ), nutritional quality (NQ) and appearance quality(AQ), granule-bound starch synthase (GBSS), soluble starch synthase (SS) and starch branching enzymes (SBE).