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Advances and Milestones of Radish Breeding: An Update

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Advances and Milestones of Radish Breeding: An Update

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Abstract

Radish is an annual herbaceous root crop, fruit, and oil crop plant belonging to the Cruciferae family. The important traits for radish breeding include high yield, early maturity, late bolting, pungency, cold-hardiness, drought resistance, heat tolerance, and soil adaptability. For successful radish production, need to the understand nature and behaviour of the flower, and very important to identify the S haplotypes of parental lines to produce F_1 hybrids based on self-incompatibility to get rid of laborious hand emasculation in radish. In radish some desirable genes are not present within varieties. Therefore, further breeding programmes depend on inter-specific and intra-specific hybridization, which has a vital role in genomic studies and crop improvement by introducing desirable agronomic characters. It is essential to acquire detailed genetic information on chromosomes and information on inheritance. Genomics is now at the core of crop improvement, and radish crop is exploited to study the underlying differences in genotypes. But some monogenic characters are improved by genetic engineering. A three-decade span following the first documented instance of genetic engineering has witnessed its application's unprecedented growth. Researchers have successfully produced transgenic radishes with various agronomic characteristics over the last decade.

Keywords: Radish, Breeding, Interspecific hybridization, Molecular breeding, Genomics, Genetic engineering

Introduction

Radish is an annual herbaceous vegetable known as *Raphanus sativus* (Jansen, 2021), and it is a diploid containing two sets of eighteen chromosomes (2n=18) (Richharia 1937). Radish belongs to the Cruciferae family, and it is eaten fresh as grated radish, a garnish, and a salad (Kaneko and Matsuzawa 1993). Radish is regularly served in eastern Asian cuisine; moreover, radish has also featured in food worldwide (Patra *et al*. 2016). There is a focus on developing high-quality radish varieties ideal for tropical and subtropical temperatures (Ebert 2013).

Additionally, breeding work has been performed on numerous agronomical traits include tolerance to pathogens, and consumption adaptability. Importance traits for radish breeding include high yield, early maturity, late bolting, pungency, cold-hardiness, drought resistance, heat tolerance, and soil adaptability

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(Kaneko and Matsuzawa 1993). There is a correlation between the radish's consistency and its amount of sugar, pungency, elaboration of the cell, water content, and pore extent (Singh *et al*. 2020).

Now days, Mass selection or pedigree methods is utilized for developing on the focus on the red globe, oval red and white forms of radish (Kaneko *et al*. 2007). . Although, the main endeavor has been to modify the radish cultivation to various growing seasons (Simard and Légère 2004).. It is essential to acquire detailed genetic information on chromosomes and information on inheritance for various genes responsible for agronomical, biochemical traits and resistance to biotic and abiotic stresses for carrying out a successful radish breeding (Kumar 2012; Dixon 2007). The studies that need to use new approaches, including chromosome or gene alteration, need to be performed (Porteus and Carroll 2005).

Marketing assessment and consumer preference are primarily associated with physical attractiveness like length, shape, size, and skin colour (Petropoulos *et al*. 2019). A primary color changes into white and different pink, red, purple, yellow, and green, but the skin is usually white. In contrast, red color radishes have a mild flavor (not as pungent) and are around 40 cm in length (Singh and Sharma 2020). The anthocyanin pelargonidin is the color causing pigment in red colours, while in purple cultivars, the cyanidin derivative is responsible for the color. Although quality-related traits may be remarkably heritable, they are often strongly influenced by cultivation methods. The swollen tap roots of radishes may be oval, tapered, or cylindrical in terms of form (Rubatzky and Yamaguchi 1997). Moreover, mechanical harvesting often includes cylindrical root cultivars (Bhardwaj *et al*. 2020). Rich in antler velvet, radish roots produce useful phytochemicals, they have cancer-preventive properties and working as a significant contributor to the taste and flavor of the Brassica vegetables (Cartea *et al*. 2008). In addition, radishes provide complex carbohydrates, dietary fibre often organic nutrients and minerals to humans, (El-Ramady et al. 2015).

Omics approaches using Next Generation Sequencing (NGS) methods provides a large amount of genomic study (Wang *et al*. 2013), enables identifying new genes and sequences and positional markers to be obtained and distributed on the chromosomes (Kim *et al*. 2019). In addition, genome-wide study results reveal the genetic causes of diverse characteristics (Kobayashi *et al*. 2020). Genome-wide exploration of suitable markers and higher-throughput genotyping will also be achieved by re-sequencing genomes (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4885859/; Kobayashi *et al*. 2020). Furthermore, there is less studyhas been published that discusses the historical milestones and technological advancements in radish breeding. As a result, we have gathered information on different elements of radish breeding and its numerous accomplishments in this section. We believe that this study will prove to be a valuable resource for vegetable breeders in the future.

Breeding Goals

the Brassicaceae family is its high nutrition content, health benefits conferred by its chemical compounds, and a significant contribution to the human being (Al-Shehbaz 2001). Root length is an important trait of radish for consumers, and preference always goes to radish on length, diameter, and color; visual indicators such as the color of the label and where it is presented are crucial to buyers' selection phase. Too many experiments have concentrated on gene mapping to classify significant color-gene associations in various vegetables for this cause (Yu *et al*. 2021). Productive radish root color is vital to radish crop productivity, the discovery and detection of large plant genes involved in radish colouring

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would aid in the advancement of color genetics (Schreiner *et al*. 2002). To date, the range of potential skin inheritance trends has been investigated extensively in radish (Yarnell 1956). Gamba *et al* reported that 609 chemical compounds within 23 categories of most studied varieties of Radish, such as Red (30%), white (13%), and black (6%) (Gamba *et al*. 2021). This study also found that nutrients, antioxidants, and phytochemicals mainly identified in roots, sprouts, and leaves, which could be considered an important part of a healthy diet (Gamba *et al*. 2021).

In addition, researchers have focused on red radish with red flesh because it contains large amounts of a natural red pigment widely used in foods, wine, and cosmetics (Jing *et al*. 2012). The uniformity of various color, size, and yield are the factors becoming a high priority goal in radish breeding (Elsayed *et al*. 2016). Radish has a wide variety of colours that affect its appearance and its nutritional quality (Zhang *et al*. 2021). However, the detection, identification, and quantification of flavonoids in multicolour radish are rarely explored. Whereas Zhang *et al*. (2020) identified that red and purple radishes contained similar anthocyanin compounds responsible for color pigmentation, including red cyanidin, callistephin, and pelargonin (Zhang *et al*. 2020). Purple ZJL contains cyanidin *o*-syringic acid, and cyanin, whereas callistephin and pelargonin contains more amount in dark red TXH. The metabolites in colored radishes that differed from SZB genes are broadly involved in the plant secondary metabolites biosynthetic pathway, such as flavonoid, flavone, isoflavonoid, and phenylpropanoid biosynthesis. This approach would be useful for cultivating important and valuable new radish varieties (Zhang *et al*. 2020).

To investigate growth, yield, and qualitative parameters of radish and of its varieties (Pusa Desi, Pusa Himani, Pusa Reshmi, Pusa Chetaki, Arka Nishant, Japanese White and IHR-1- 1) were used in the study. It was found that total fresh weight of plant (190.06 g to 226.60 g) was observed in variety Arka Nishant, whereas minimum in variety Pusa Desi (Dongarwar *et al*. 2018). These results explain anthocyanin synthesis in radish and provide potential genetic clues for improving anthocyanins in radish roots (Giusti and Wrolstad 1996). A pathogen could damage harassment of yield, and in root color, these varieties would not be preferred for the consumer. Fusarium wilt (FW) is vascular wilt soil-borne disease and caused by fungal pathogen *Fusarium oxysporum* f. sp. Raphanin, causes severe yield losses in the production of radish (Bosland 1988). The most emerging effective method to control the FW by using resistant varieties in crop improvement (Fita *et al*. 2015). Fusarium- resistance is highly studied among 'Motohashi-' or 'Kuroba-mino' lines of Minowase variety and Tosai' is the strongest line among Nerami varieties (Hida and Ashisawa 1985). it was found that providing good quality of seed and accurate quantity of seed would increase the yield of radish (Baksh *et al*. 2006). To treat several diseases, bioactive compounds in *Raphanus sativus* (radish) is being studied, and therefore, radish has attracted scientific attention due to its nutritional and phytochemical composition, which reduces the risk of developing many cancers and cardiovascular diseases (Lima *et al*., 2008). Further, the important goals are provided in the Fig. 1.

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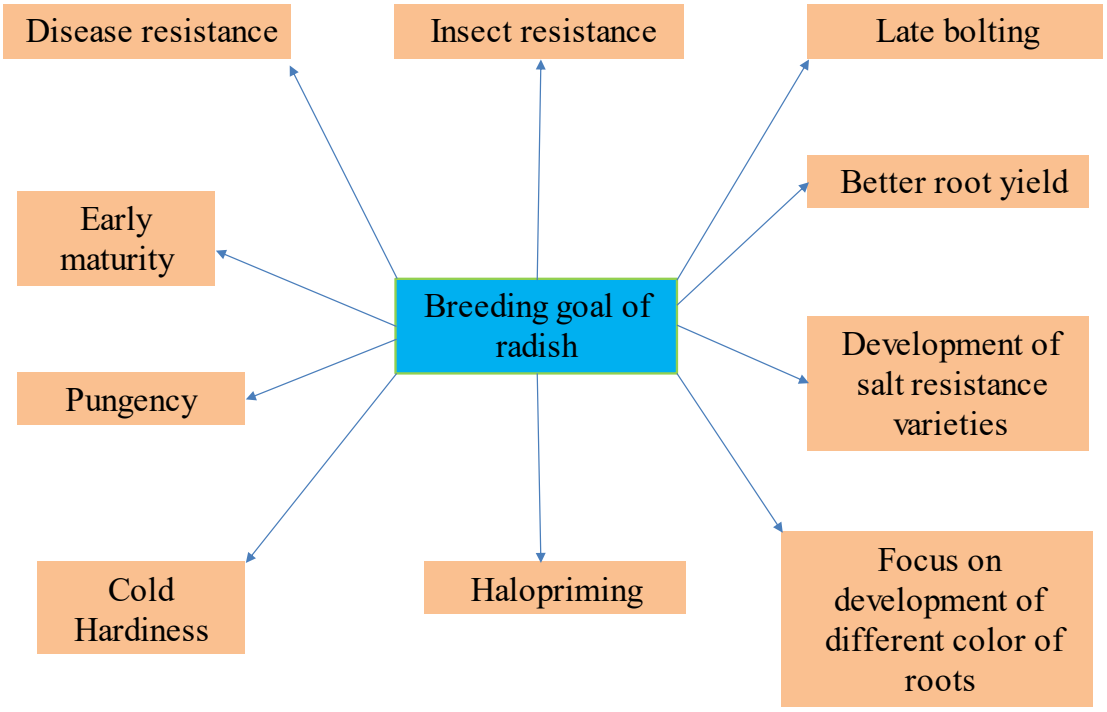

Fig. 1. Breeding goals of radish (*Raphanus sativus* L.)

Moreover, salinization is considered as one of the major soil pollutions in the environment affecting plant growth and soil fertility globally (Shrivastava *et al*. 2015) This scenario alarms an urgent need to enrich the soil or to identify stress-tolerant plants. It is reported that antioxidant enzymes (HOD-Hydrogen Peroxide; SOD-Superoxide; LOD-Lipid Peroxidation; CAT-Catalase) plays a major role in reducing the effects of salts in plants by monitoring the oxidative stress in them (Vishnu-Priya *et al*. 2020). To study the salt tolerance of Japanese wild radishes called 'Hamadaikon' (*Raphanus sativus* f. raphanistroides Makino) and its characteristics like seed germination, plant height, root length and fresh weight were examined under the salinity condition, and it was found that higher germination and growth in NaCl were shown at 25° C than those at 20° C (Sugimoto 2009). Hence, the wild radishes could be considered for the salt tolerance breeding. Moreover, halopriming is a seed priming technique in which the seeds were soaked in various salt solutions enhances germination and seedling emergence uniformly under adverse conditions. The effect of halopriming on germination, initial growth and development of radish under salt stress conditions was studied. It was found that the best effect was achieved by priming with CaCl2 for germination characteristics and vigour and with KCl for initial development (Kanjevac *et al*. 2021).

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Botany of radish

Radish (*Raphanus sativus* L.) is an entomophilous flower classified as an allogamous plant (Bateman 1955). Regular flowering appears from three florets on the tip of each branch of the panicle and each flower is effective in producing a pod upto1 to 3 inches long and consists of one to six seeds (Bailey 1949). The radish flowers open in the morning with the fresh corolla and remain until the next day (Kremer 1945). Kremer also reported that pollen receptivity of the flower limits upto few hours a day. Its flowers are 1.5–2 cm in width, whitish to pinkish and purplish colour with purple veins, and have four erected sepals and clawed petals, six stamens and 3–4 cm long style (Nishio 2017; Nayik *et al*. 2020). Siliqua or seedpod, a type of seed capsule of radish, is 1.5 cm wide and 3 to 7 cm long, consisting of 6–12 seeds/pod with a long conical seedless beak (Singh and Sharma 2020). The inflorescence of radish is a typical elongated, erected, an oblong raceme of Cruciferae (Kercher and Conner 1996; Gopalakrishnan 2007). The study of pollination of radish was carried out by Crane and Mather (1943), Kremer (1945), and Radchenko (1966). The main objective of the investigation was the cross-pollination of radish by Crane and Mather (1943), who found that the 'Icicle' and 'Scarlet Globe' cvs were self-incompatible pollinated with the help of honeybees (Young and Stanton 1990). The studies indicated that the seed yield is greatly influenced by the number of honeybees striking the radish flowers (Kremer 1945). Radchenko (1966) also reported that honeybees were the main pollinators of radish flowers, approximately 77 to 99 percent in total, increasing the crop yield by 22 percent and enhancing the seed quality. Therefore, radish is considered as almost entirely insect-pollinated (Jones and Rosa 1928). During fruit maturation, the color of seeds is somewhat yellow that turns reddish-brown with age (Kalia 2005; Lim 2015). The mature radish leaves are alternate and arranged in a rosette pattern and have a lyrate shape that is set apart pinnately with an enhanced terminal lobe and minor lateral lobes (Gopalakrishnan 2007). A longer root form, including oriental radishes, daikon or mooli and winter radishes, grows up to 60 cm (24 in) long with foliage about 60 cm (24 in) high with a spread of 45 cm (18 in) (Brickell 1992).

S haplotype

Radish is a self-incompatibility crop exhibit the high heterosis, production of F_1 hybrids based on self-incompatibility are desired to get rid of laborious hand emasculation in radish (Nishio and Sakamoto 2017). The main aim of a plant breeder is to identifythe breeding lines of S haplotypes. The plant breeder can avoid the cross incompatibility of the parental lines (Wang 2018). The S haplotype of each parental line need to show the compatible reaction between parental lines, therefore for producing F_1 hybrid breeding, it is very important to identify the S haplotypes of parental lines (Nou *et al*. 1993; Ockendon 2000). The abundance of S haplotype determines a specific S haplotype by using traditional methods including test cross method, pollination, isoelectric focusing, immunoblot analysis, and the pollen tube fluorescence analysis (Ruffio-Châble *et al*. 1997; Georgia *et al*. 1982; Martin 1959). The S alleles are highly variable in S haplotype (Ockendon 2000). Moreover, Nikura and Matsuura identified 37 alleles in Radish (Nikura and Matsuura 2001).

Several S haplotypes in *Raphinus sativus* were identified based on polymorphism in SLG, SRK and SCR/SP11 sequence and S haplotypes are numbered as S-1, S-2, S-3, etc (Nikura and Matsuura 2001; Takeshi and Sakamoto 2017). Although radish belongs to a genus different from Brassica, nucleotide sequences of SP11, SRK, and SLG alleles of radish and Brassica are intermingled in phylogenetic trees of SP11, SRK, and SLG, respectively, indicating that diversification of these alleles predates speciation of

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these genera (Wang *et al*. 2018). SP11, SRK, and SLG alleles of some S haplotypes in radish are highly similar to those of some S haplotypes in Brassica, and one S haplotype in radish has been revealed to have the same recognition specificity as that of one S haplotype in B. rapa (Wang *et al*. 2018). Comparison of nucleotide sequences of SP11 and SRK alleles and recognition specificities between similar S haplotypes of radish and Brassica may provide valuable information for understanding the molecular structures of SP11 and SRK proteins. However, researchers' numbering of S haplotypes in radish varies, and nucleotide sequence information on S haplotypes is thus confusing (Nishio and Sakamoto 2017).

Besides, analysis of SLG and SRK is utilized to identify S haplotype in Raphanus and Brassica by the using methods polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) (Lim *et al*. 2002; Nishio *et al*. 1997; Okamoto *et al*. 2004). However, the own limitation of PCR-RFLP, first, it is difficult to design a universal primer that can amplify SLG and SRK alleles; second, the presence of multiple genes homologous to the SLG or SRK genes in Brassicaceae plants aggravates PCR amplification of specific SLG or SRK alleles (Lalonde *et al*. 1989; Boyes *et al*. 1991; Kumar and Trick 1993; Suzuki *et al*. 1997; Wang 2018).

Additional advanced radish cultivars (cultivars with improved yield and higher quality) were also produced by the Ogura CMS method to assist in radish hybrid (Bhardwaj *et al*. 2020), this variety shows such as bulk selection, mixed mass pedigree selection, or bud pollination will take eight to twelve years to produce a new variety, new varieties must be produced from other genetic means (Cox and Beaton 2020).

Interspecific hybridization

Inter-specific and intra-specific hybridization has an important role in genomic study and crop improvement by introducing desirable agronomic characters and specific traits such as disease, insects and stress resistance from wild species to cultivated ones (Bowely and Taylor 1987;Kaneko and Woo 2014). Jin *et al*, 2020, observed thatthe crosses between radish, turnip, and Chinese kale were made to produce F_1 hybrids (Jin *et al*. 2020). The study indicated that the average podding rate of the cross between radish and turnip (67.03%) was much higher than that of the reciprocal cross between turnip and radish (55.04%) (Jin *et al*. 2020), and it was also reported that the average seed-setting rate and hybrid acquisition rate of the radish and turnip based on cross pattern (e.g., 2.25% and 0% respectively), however, seed production of the F_1 hybrids and their F_2 progeny was up to 0.4% and 2%, respectively, as compared to wild radish (Darmency *et al*. 1998). Therefore, the study indicated that there was a low hybridization affinity between radish and Chinese kale, but incompatibility still prevailed (Jin *et al*. 2020).

Similarly, the radish-wild mustard interspecific hybrid was studied. It was found that production was higher with radish pollen competition, i.e, 42 and 3 interspecific hybrid seeds per 1000 seeds were observed (Eber *et al*. 1998). Another study by Peter *et al* indicated that the modified flower culture method is the best method for hybridization between radish and (transgenic) oilseed rape (*Raphanobrassica* hybrids) without labour-intensive *production vitro* ovule or embryo rescue techniques. This is a potential approach for breeding programs by introducing useful radish genes, e.g., nematode resistance genes, into oilseed rape (Metz *et al*. 1995). Moreover, clubroot is a common disease of cabbages, cauliflower, radishes, turnips, and other plants of the family Brassicaceae caused by *Plasmodiophora brassicae* (Bhattacharya *et al*. 2014). Radish is a close relative of the brassica family, and it was found that a synthesized allotetraploid *Brassicoraphanus* (RRCC, 2n = 36) between *R. sativus* cv. HQ-04 (2n = 18,

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RR) and *Brassica oleracea* var. *alboglabra* (L.H Bailey) (2n = 18, CC) proved resistant to multiple clubroot disease pathogen *P. brassicae* causing club root disease (Zhan *et al*. 2017).

However, the spontaneous hybridization event between *Brassica napus* (oilseed rape) and *Raphanus raphanistrum* (wild radish) was screened, and it was found that hybrids with wild radish as the seed parent contributes for herbicide resistance belonging to rape. Another study by Darmency *et al*, 1998, indicated that wild radish in an oilseed rape field produced as many as three interspecific hybrids per 100 plant and was the first-ever such report of such a spontaneous event (Darmency *et al*. 1998).

Molecular Markers to QTLian Breeding

Several economically important traits in radish are being identified, these traits are yield, insect resistance and disease resistance (Kaneko and Matsuzawa 1993). = Yield is a complex trait governed by the polygenic characters, to identifythese traits using conventional breeding/traditional breeding is difficult because these traits depend on phenotypic expression and have environmental and genotypic interaction. These problems are overcome by the new tool molecular breeding, identification of quantitative trait is being utilized with the help of DNA markers (Kaneko *et al*. 2007) and linkage mapping (Xu *et al*. 2012), there are several DNA marker are being utilized in breeding programme such as restriction fragment length polymorphism (RFLPs), random amplified polymorphism dna (RAPD), simple sequence repeats (SSRs), single nucleotide polymorphism (SNPs) (Muminović *et al*. 2005, Tsuro *et al*. 2008, Xu *et al*. 2012, Budahn *et al*. 2009, Hashida *et al*. 2013). Molecular markers such as RAPD have been used to establish the origin of hortensis var. sativus and var. niger, which formed from distinct progenitors and came from different sources (Curtis 2011). Several Asian varieties show greater skin and flesh color, size, length, and weight of roots, var. hortensis' genetic variability is also not a surprise.

Moreover, Lee *et al.* (2021) performed genome-wide association studies (GWAS) using genotyping-by-sequencing (GBS) for identifing FW resistance loci followed by Phenotypic studies conduction (Lee *et al*. 2021). The GWAS analysis identified 44 single nucleotide polymorphisms (SNPs) and twenty putative candidate genes that were significantly associated with FW resistance. A total of four QTLs were identified from F_2 population derived from a FW resistant line and a susceptible line, among these one of which was co-located with the SNPs on chromosome 7. These markers are emerging tools for molecular breeding programs and marker-assisted selection to develop FW resistant varieties of *R. sativus* (Lee *et al*. 2021). Moreover, for identification of disease Fusarium wilt, Yu *et al*. 2013, constructed a genetic linkage map on the F_2 population, they observed total 8 loci conferring FW resistance that were distributed on 4LGs, namely 2, 3, 6 and 7 of the Raphanus genome. Synteny analysis using the linked markers QTL showed homology to A. thaliana chromosome 3, which contains disease-resistance gene clusters, suggesting conservation of resistance genes between them. The list of important QTLs is identified in the radish and their location is provided in the Table 1.

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Table 1. List of QTLs identified for important traits in radish.

Locus ID	Locus Name	Trait	Cross	Population	Marker Name	Max LOD	References
t3726.T000001	qFW1	Fusarium wilt resistance	835 x B2	F2	nu_mBRPGM1376	3.72	Yu *et al.* 2013
t3726.T000002	qFW1	Fusarium wilt resistance	835 x B2	F2	ACMP0606	4.34	Yu *et al.* 2013
t3726.T000003	qFW1	Fusarium wilt resistance	835 x B2	F2	nu_mBRPGM1208	9.42	Yu *et al.* 2013
t3726.T000004	qFW1	Fusarium wilt resistance	835 x B2	F2	ACMP0357	10.08	Yu *et al.* 2013
t3726.T000005	qFW1	Fusarium wilt resistance	835 x B2	F2	nia032a	3.31	Yu *et al.* 2013
t3726.T000006	qFW2	Fusarium wilt resistance	835 x B2	F2	nu_mBRPGM1376	3.29	Yu *et al.* 2013
t3726.T000007	qFW3	Fusarium wilt resistance	835 x B2	F2	ACMP0590	5.62	Yu *et al.* 2013
t3726.T000008	qFW4	Fusarium wilt resistance	835 x B2	F2	nu_mBRPGM0432	3.76	Yu *et al.* 2013
t3726.T000009	qFW5	Fusarium wilt resistance	835 x B2	F2	nu_mBRPGM0432	4.84	Yu *et al.* 2013
t3726.T000010	qFW6	Fusarium wilt resistance	835 x B2	F2	nu_mBRPGM0432	4.23	Yu *et al.* 2013
t3726.T000011	qFW7	Fusarium wilt resistance	835 x B2	F2	ACMP0606	7.94	Yu *et al.* 2013
t3726.T000012	qFW8	Fusarium wilt resistance	835 x B2	F2	ACMP0606	8.84	Yu *et al.* 2013

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t3726.T000013		Root shape (length/width)	Huang-he hong wan x Utsugi-gensuke	F2	BRMS-303-2	2.42	Tsuro *et al.* 2008
t3726.T000014		Thickening	Huang-he hong wan x Utsugi-gensuke	F2	ACA/CTT5	3.03	Tsuro *et al.* 2008
t3726.T000015		Thickening	Huang-he hong wan x Utsugi-gensuke	F2	AAG/CTA12	4.51	Tsuro *et al.* 2008
t3726.T000016		Root shape (length/width)	Huang-he hong wan x Utsugi-gensuke	F2	ACA/CTG5	2.88	Tsuro *et al.* 2008
t3726.T000017		Red pigmentation	Huang-he hong wan x Utsugi-gensuke	F2	SLG	9.58	Tsuro *et al.* 2008
t3726.T000017		Red pigmentation	Huang-he hong wan x Utsugi-gensuke	F2	SLG	9.58	Tsuro *et al.* 2008
t3726.T000018		CR (clubroot resistance), Crs1 (clubroot resistance locus of Rap	Koga-benimaru x Utsugi-gensuke	F2	BN142	12.64	Kamei *et al.* 2010
t3726.T000019	qRCd1	RCd (mg kg-1), root Cd concentration	Nau-Dysx x Nau-Yh	F2	Ni2E05_525	5.24	Xu *et al.* 2012
t3726.T000019	qRCd1	RCd (mg kg-1), root Cd concentration	Nau-Dysx x Nau-Yh	F2	Ni2E05_525	5.24	Xu *et al.* 2012
t3726.T000020	qRCd4	RCd (mg kg-1), root Cd concentration	Nau-Dysx x Nau-Yh	F2	BRMS129_510	4.36	Xu *et al.* 2012

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t3726.T000020	qRCd4	RCd (mg kg-1), root Cd concentration	Nau-Dysx x Nau-Yh	F2	BRMS129_510	4.36	Xu *et al.* 2012
t3726.T000021	qRCd6	RCd (mg kg-1), root Cd concentration	Nau-Dysx x Nau-Yh	F2	EM6fc3_331	5.28	Xu *et al.* 2012
t3726.T000021	qRCd6	RCd (mg kg-1), root Cd concentration	Nau-Dysx x Nau-Yh	F2	EM6fc3_331	5.28	Xu *et al.* 2012
t3726.T000022	qRCd9	RCd (mg kg-1), root Cd concentration	Nau-Dysx x Nau-Yh	F2	EM5me6_286	23.64	Xu *et al.* 2012
t3726.T000022	qRCd9	RCd (mg kg-1), root Cd concentration	Nau-Dysx x Nau-Yh	F2	EM5me6_286	23.64	Xu *et al.* 2012
t3726.T000023	qRDW5	RDW (g), root dry weight	Nau-Dysx x Nau-Yh	F2	BRMS058_550	7.28	Xu *et al.* 2012
t3726.T000024	qRDW6	RDW (g), root dry weight	Nau-Dysx x Nau-Yh	F2	NAUrp705_644	3.96	Xu *et al.* 2012
t3726.T000025	qRDW9	RDW (g), root dry weight	Nau-Dysx x Nau-Yh	F2	EM3me6_291	3.58	Xu *et al.* 2012
t3726.T000026	qRL1	RL (cm), root length	Nau-Dysx x Nau-Yh	F2	Na10F06_545	3.38	Xu *et al.* 2012
t3726.T000027	qRL3.1	RL (cm), root length	Nau-Dysx x Nau-Yh	F2	RamRM24-568_618	3.26	Xu *et al.* 2012
t3726.T000028	qRL3.2	RL (cm), root length	Nau-Dysx x Nau-Yh	F2	PM2fc8_314	3.64	Xu *et al.* 2012
t3726.T000029	qRL5	RL (cm), root length	Nau-Dysx x Nau-Yh	F2	NAUrp782_643	4.89	Xu *et al.* 2012
t3726.T000030	qRL7	RL (cm), root length	Nau-Dysx x Nau-Yh	F2	EM4odd48_365	4.06	Xu *et al.* 2012

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t3726.T000031	qSCd1	SCd (mg kg-1), shoot Cd concentration	Nau-Dysx x Nau-Yh	F2	NAUrp362_706	4.37	Xu *et al.* 2012
t3726.T000031	qSCd1	SCd (mg kg-1), shoot Cd concentration	Nau-Dysx x Nau-Yh	F2	NAUrp362_706	4.37	Xu *et al.* 2012
t3726.T000032	qSCd3	SCd (mg kg-1), shoot Cd concentration	Nau-Dysx x Nau-Yh	F2	EM16ga18_383	7.64	Xu *et al.* 2012
t3726.T000032	qSCd3	SCd (mg kg-1), shoot Cd concentration	Nau-Dysx x Nau-Yh	F2	EM16ga18_383	7.64	Xu *et al.* 2012
t3726.T000033	qSDW2	SDW (g), shoot dry weight	Nau-Dysx x Nau-Yh	F2	NAUJKC19_565	4.74	Xu *et al.* 2012
t3726.T000034	qSDW6	SDW (g), shoot dry weight	Nau-Dysx x Nau-Yh	F2	Na10F08_665	3.78	Xu *et al.* 2012
t3726.T000035	qSDW9	SDW (g), shoot dry weight	Nau-Dysx x Nau-Yh	F2	OI14E06_720	4.62	Xu *et al.* 2012
t3726.T000036	qSH2	SH (cm), shoot height	Nau-Dysx x Nau-Yh	F2	EM16ga18_384	4.25	Xu *et al.* 2012
t3726.T000037	qSH5	SH (cm), shoot height	Nau-Dysx x Nau-Yh	F2	RGA12F12R_300	3.64	Xu *et al.* 2012
t3726.T000038	qTDW1.1	TDW (g), total dry weight	Nau-Dysx x Nau-Yh	F2	RamM2-706_616	3.54	Xu *et al.* 2012
t3726.T000039	qTDW1.2	TDW (g), total dry weight	Nau-Dysx x Nau-Yh	F2	PM2em11_367	5.6	Xu *et al.* 2012
t3726.T000040	qTDW5	TDW (g), total dry weight	Nau-Dysx x Nau-Yh	F2	PM17em10_327	4.43	Xu *et al.* 2012
t3726.T000041	qTDW6	TDW (g), total dry weight	Nau-Dysx x Nau-Yh	F2	NAUrp586_752	3.14	Xu *et al.* 2012

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t3726.T000042	qTDW7	TDW (g), total dry weight	Nau-Dysx x Nau-Yh	F2	PM18odd44_362	4.84	Xu *et al.* 2012
t3726.T000043	qTDW9	TDW (g), total dry weight	Nau-Dysx x Nau-Yh	F2	PM36em8_423	6.2	Xu *et al.* 2012
t3726.T000044	Hs1_Rph	BCN-resistance, resistance against the beet cyst nematode (H. sc	Pegletta x Siletta Nova	F2	E41M59-297	22.6	Budahn *et al.* 2009
t3726.T000045		PS, Plant shape	rat-tail radish x Haru-S	RIL	RsHH019	3.8	Hashida *et al.* 2013
t3726.T000046		MW, Main root weight (g)	rat-tail radish x Haru-S	RIL	REL-13	6.9	Hashida *et al.* 2013
t3726.T000047		Pubescence	rat-tail radish x Haru-S	RIL	RM_1	10.1	Hashida *et al.* 2013
t3726.T000048		MW, Main root weight (g)	rat-tail radish x Haru-S	RIL	RES-1	11.7	Hashida *et al.* 2013
t3726.T000049		WW, Whole plant weight (g)	rat-tail radish x Haru-S	RIL	AtSTS-1015	3.8	Hashida *et al.* 2013
t3726.T000050		Pubescence	rat-tail radish x Haru-S	RIL	RsSR104	13.6	Hashida *et al.* 2013
t3726.T000051	GSL-QTL-1	4MTB-GSL contents, 4-methylthio-3-butenyl glucosinolate contents	TBS x AZ26H	F2	RS2CL6432s	5.87	Zou *et al.* 2013
t3726.T000052	GSL-QTL-1	4MTB-GSL contents, 4-methylthio-3-butenyl	TBS x AZ26H	F2	S2CL4585s	3.62	Zou *et al.* 2013

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		glucosinolate contents					
t3726.T000053	GSL-QTL-1	4MTB-GSL contents, 4-methylthio-3-butenyl glucosinolate contents	TBS x AZ26H	F2	RS2CL3356s	3.85	Zou *et al.* 2013
t3726.T000054	GSL-QTL-1	4MTB-GSL contents, 4-methylthio-3-butenyl glucosinolate contents	TBS x AZ26H	F2	RS2CL4290s	7.36	Zou *et al.* 2013
t3726.T000055	GSL-QTL-2	4MTB-GSL contents, 4-methylthio-3-butenyl glucosinolate contents	TBS x AZ26H	F2	RS2CL6432s	19.1	Zou *et al.* 2013
t3726.T000056	GSL-QTL-3	4MTB-GSL contents, 4-methylthio-3-butenyl glucosinolate contents	TBS x AZ26H	F2	RS2CL6594s	5.62	Zou *et al.* 2013
t3726.T000057	GSL-QTL-4	4MTB-GSL contents, 4-methylthio-3-butenyl glucosinolate contents	TBS x AZ26H	F2	RS2CL6594s	1.54	Zou *et al.* 2013
t3726.T000058	GSL-QTL-5	4MTB-GSL contents, 4-methylthio-3-butenyl glucosinolate contents	TBS x AZ26H	F2	S2CL4585s	5.19	Zou *et al.* 2013

Moreover, identification of root shape and red pigmentation is done by the Tsuro *et al*. 2008, they observed that three quantitative trait loci for root shape, namely, LG3, LG8 and LG9, two QTLs for root diameter, namely LG4 and LG8 and one for red pigmentation is identified with the help of using AFLP, SSR and SLG-CAPS (Tsuro *et al*. 2008). Kamei *et al*. 2010, constructed a genetic linkage map using AFLP and SSR markers and concluded that CR is governed by the single gene or closely linked gene loci namely,

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Crs1, Crs2 and Crr3 (Kamei *et al*. 2010). A genetic map was constructed using an F_2 population by using markers SRAP, RAPD, SSR, ISSR, RAMP and RGA markers, and they found that a novel QTL qRCD9 is responsible for controlling root CD (Xu *et al*. 2012). Resistance against cyst nematode (*Heterodera schachtii*) was identified by using RAPD, dpRAPD, AFLP and SSR markers (Budahn *et al*. 2008). To identify quantitative traits in radish for morphological characters namely, ovule number per silique, seed number per silique, plant shape, pubescence, whole plant weight (g), upper part weight (g), whole root weight (g), main root weight (g) using recombinant inbred lines, they identified 8 and 10 quantitative traits in 2008 and 2009 respectively (Hashida *et al*. 2013). In the identified QTL regions, nine SNP markers were newly produced. Nucleotide sequences and expression of these genes suggested their possible function in 4MTB-GSL biosynthesis in radish roots. (Zou *et al*. 2013). Whereas Fan *et al.* (2020) discovered that the R2R3-MYB transcription factor responsible for anthocyanin pigment 2 (PAP2) production is located on chromosome 2. The amino acid sequence encoded by the RsPAP2 gene was entirely distinguishable from other previously published different RsMYB genes responsible for the red skin color of radish.

Genomics

Genomics is now at the core of crop improvement, and radish crop has been exploited to study the underlying differences in genotypes. The rapid development of genomic data boosted the discoveries regarding the genetic basis of plant traits, such as increased yield, flowering, or disease resistance (Tuberosa and Salvi 2006). A variety of systematic studies of the radish have investigated their genomes' arrangement and the reorganization of chromosomes during polyploidy events (Li *et al*. 2011), of which draft genomic sequences has been assembled (Kitasiba *et al*. 2012). Another study reported an Asian radish cultivar, WK10039 which was sequenced entirely by combining 454, Illumina, and PacBio sequencing systems and bacterial artificial chromosome clones obtained through end sequencing, was fully sequenced by using the end sequencing method and sequencing equipment from the ABI firm (Jeong *et al*. 2016), over the last decade, a variety of genomic studies on the cultivated radish have been performed (Jeong *et al*. 2014). Moreover, a chromosome-scale genome assembly (rs1.0) of WK10039, an Asian radish cultivar, and compared it to assemblies documented previously was constructed (Mitsui et al. 2015). It revealed more details than those previously recorded (having greater coverage of the genome, a greater number of contigs, and chromosome anchoring) (Lee et al. 2018). However, Radish Base is a genomic and genetic database that currently contains radish mitochondrial genome sequences (Shen *et al*. 2013). This database presently includes the mitochondrial genomes of two newly sequenced radish species, one from the normal cytoplasm and the other from the male-sterile cytoplasm of Ogura (Tanaka *et al*. 2012). The previous study published the bioinformatics analysis in radish and identified 20 *COL* transcription factors in the radish genome among 54,357 coding genes (Hu *et al*. 2018). Every *COL* gene in the 'Aokubi daikon' cultivar was matched with the corresponding *COL* gene in the 'kazusa' cultivar. A total of 20 radish *COL* genes were also searched in the cultivar 'WK10039' (Hu *et al*. 2018). Besides, in radish genome, thirty-five unique RsOFPs and five RsOFP-likes (with no/partial OVATE domain) were identified by BLASTP and analysis of exon-intron organization revealed that most genes were intron-less contains maximum coding sequences in the genome (Hu *et al*. 2018)

In addition, *R.sativus* inbred line XYB36-2, a 119.75 Gb of whole-genome shotgun sequences were performed using a HiSeq2000 system (Xiaohui *et al*. 2015). On the basis of 17-mer analysis, the estimated size of genome came out to be 530 Mb. A 387.73 Mb was assembled into 44 820 high-quality scaffolds using SOAP denovo (Li *et al*. 2011) and SSPACE (Boetzer *et al.* 2011). The assembly in this study showed

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excellent results with fosmid clones (98.86% covered), the assembly showed a much higher quality than the draft genome of *R. raphanistrum* (254 Mb contigs) (Moghe *et al*. 2014) and two assemblies (116.0 and 179.8 Mb) of *R. sativus* 'Aokubi' (Kitashiba *et al*. 2014,) which was released previously. After de novo assembly of the 'Okute-Sakurajima' genome, an estimated haploid genome size of 498.5 Mb was found. The de novo assembly showed a substantially heterozygous genome (Shirasawa *et al*. 2020). Subsequent long-read sequencing produced 36.0 Gb data (60.7 coverage of the estimated genome size) in 2.3 million reads with N50 length of 29.1 kb. After two rounds of data polishing, the long-read assembly consisting of 504.5 Mb primary contigs (including 1,437 sequences with an N50 length of 1.2 Mb) and 263.5 Mb alternative contigs consist of the other haplotypes with different alleles, also known as haploid sequences (including 2,373 sequences with an N50 length of 154.6 kb) (Shirasava *et al*. 2020).

Study performed on the radish genome after polyploidy has shown fundamental information about the radish genome production and evolution, which provides valuable insights into radish genetics and breeding (Yu *et al*. 2017). The detailed data and genomic methods obtained through these investigations support a greater understanding of the radish triplicated genome composition. Additionally, these methods help radish breeding by promoting the usage of marker-assisted collection, comparative genomic studies, and the transmission of knowledge from the reference data to other radish accessions (Varshney *et al*. 2021). Consequently, a portal that is home to considerable quantities of genomic information and various links to specific genome analysis methods is extremely valuable in radish research and breeding.

Genetic Engineering

Genetic engineering has pvotal role in agriculture by improving characters in the crops and satisfying the need of poor nourished countries. The Developments in gene technology and metabolic engineering systems accelerated the production of useful germplasms (Wang *et al*. 2017). The Progress is being achieved in plants methods by improving the traits; researchers have successfully produced transgenic radishes with various agronomic characteristics (Curtis and Nam 2001). Some radish varieties exhibit favorable traits, including improved yield, and transfer of these characteristics on to the host plant (Tzfira and Citovsky 2008; Lacroix and Citovsky 2019).

Gene transmission is done with the help of pathogen, known as agrobacterium, is extensively used as a technique for plant hairy root lines, which appear to yield better than other forms of root systems (Ali *et al*. 2008). Herbaceous hairy roots have advantageous due to their longevity, pace of growth, and capacity to assist plants in growing from the root up (Gelvin 2009). The hairy roots are produced in nutrient solution with the help of growing agrobacterium that contain unusual properties, including biochemically and bio-transforming different metabolites. It is best to use Agrobacterium to produce secondary metabolites since they help to enhance the growth regulators (Giri and Narasu 2000). Working on the hairy roots, it can be discovered new sources of natural compounds (Berkov *et al*. 2003),

In addition, chromosomal disruption or amplification may affect the fertility of cultured plants. Antibiotics, herbicides, metabolic analogues, and non-toxic agents all facilitate transformed cell for survival. Kanamycin and hygromycin B hamper radish regeneration (Curtis *et al*. 2004).

Recent advancements in plant biotechnology indicate that radish could be genetically modified via a process called "floral-dipping." This technique involves co-suppression of the photoperiodic gene

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GIGANTEA in radish and contributes to the plant's ability to delay bolting and blooming. It can be used for boosting a crop's medicinal value (Curtis 2003). The prospects for improving transformation efficiency and selecting new traits for generating late-flowering radish are published (Curtis *et al*. 2011). In 2001, it was demonstrated that plants derived from plants dipped into an Agrobacterium suspension containing both the beta-glucuronidase (gusA) gene and the herbicide resistance gene (bar) between the flanking T-DNA border sequences could be used to generate transgenic radish (*Raphanus sativus* L. longipinnatus Bailey). (Curtis and Nam 2001). At the end, Southern blotting results revealed that both the gusA and bar genes integrated into the genome of transformed plants and segregated as dominant Mendelian traits (Curtis and Nam, 2001). A study revealed that The *RHA2b* gene from radish encodes a transcription factor involved in abscisic acid (ABA) signal transduction and is responsible for seed dormancy and preharvest sprouting (Li *et al*. 2019). The study performed the experimentation in which The *RsRHA2b* gene was cloned and transferred into Zhengmai 9023 via Agrobacterium-mediated stem apex transformation (Li *et al*. 2019). The agrobacterium-mediated transformation became a more appropriate method for genetic transformation (Hayta *et al*. 2019). The use of adventitious shoot growth on hypocotyl explants for Agrobacterium-mediated radish genetic transformation was investigated using transgenic radish (Raphanus sativa L., cv. Jin Ju Dae Pyong) grown on Murashige and Skoog medium (Cho *et al* 2008). Besides, northern blot results revealed the GUS gene transcript was detected in few regenerated plants hence, confirming genetic transformation (Cho *et al*. 2008).

In addition, the techniques available for introducing pharmaceutical proteins into radish for on- site delivery of edible proteins into it is discussed by Curtis in his study (Curtis 2003). The concerns of releasing transgenic radish to the field in pollen-mediated gene transfer have also been explored. Risks that might exist and the introduction of transgenic radish to the field are sometimes brought up in discussions about transgenic crops (Curtis and Nam 2001; Rissler *et al*. 1996; Snow and Palma 1997).

Conclusion and Future Directions

The production of yields, early maturity and late bolting, pungency, cold hardiness, drought resistance, heat tolerance, and soil adaptability are just a few of the essential radish breeding traits. The radish genome contains self-incompatibility alleles, allowing for the generation of F_1 hybrids without the labour-intensive and without hand emasculation required in radish. When generating F_1 combinations, it is critical to determine the S haplotypes of the parental lines to avoid hand emasculation. For successful production of radish yield, inter- and intra-specific hybridization are vital to genetic research and crop improvement because they enable the introduction of desirable agronomic traits into the population. Collecting complete genetic data on chromosomes as well as information on inheritance is critical. To better understand and forecast resistance, yield characteristics, and fruit quality, researchers must understand the regulatory factors that synchronize at various developmental stages for each of the attributes discussed. It remains necessary to develop a robust and long-lasting strategy for plant disease resistance, which is currently under consideration. This is because diseases are capable of evading resistance by generating novel bacterial strains.

Due to the completion earlier this year of large-scale sequencing of the radish genomes, scientists will now have precise information on disease resistance genes for a variety of diseases and genes encoding critical biochemical properties of the plant. Speed breeding is one such strategy; as the cost of genome sequencing continues to decline, RAD-sequencing and DNA microarrays will become more common,

enabling faster genome mapping and tagging of new quantitative trait loci. These quantitative trait loci (QTLs) may incorporate resistance into high-yielding radish genotypes and combine them with significant resistance genes to increase the number of resistant radish genotypes. Additionally, GWAS (genome-wide association studies) can map characteristics to specific candidate genes on a genome-wide scale to improve crop production and quality in radish. The discovery of significant genetic and metabolic diversity paves the way to develop controlled harvest variations in agriculture and genetic enhancement via breeding.

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References

Ali MA, Azeem F, Abbas A, Joyia FA, Li H, Dababat AA. 2017. Transgenic strategies for enhancement of nematode resistance in plants. Front. in plant sci. 750.

Al-Shehbaz IA. 2001. Brassicaceae (mustard family). *e LS*.

Bailey LH. 1949. Manual of cultivated plants. *Manual of Cultivated Plants.*, (Rev. Edn.).

Bakhsh KH, Ahmad BA, Gill ZA, Hassan SA. 2006. Estimating indicators of higher yield in radish cultivation. Int. J. Agric. Biol. 6: 783-789.

Bateman AJ. 1955. Self-incompatibility systems in angiosperms. *3. Cruciferae* (No. RESEARCH).

Berkov S, Pavlov A, Kovatcheva P, Stanimirova P, Philipov S. 2003. Alkaloid spectrum in diploid and tetraploid hairy root cultures of Datura stramonium. Z Natur f. 58: 42–46.

Bhardwaj RK, Kumari R, Vikram A. 2020. Efficient Methods for the Improvement of Temperate Root Vegetables. In Accel. Plant Breeding. 155-196).

Bhardwaj RK, Kumari R, Vikram A. 2020. Efficient Methods for the Improvement of Temperate Root Vegetables. In *Accel. Plant Breed. 2*: 155-196.

Bhattacharya I, Dutta S, Mondal S, Mondal B. 2014. Clubroot disease on Brassica crops in India. Can. J. of Plant Path. 36: 154-160.

Boetzer M, Henkel CV, Jansen HJ, Butler D, Pirovano W. 2011. Scaffolding pre-assembled contigs using SSPACE. Bioinf. *27*: 578-579.

Bosland PW. 1988. Fusarium oxysporum, a pathogen of many plant species. *Genet. of Pl. Pathog. Fun. 6*: 281-288.

Bowley SR, Taylor NL. 1987. Introgressive hybridization.

Boyes DC, Nasrallah JB. 1993. Physical linkage of the SLG and SRK genes at the self-incompatibility locus of Brassica oleracea. Mol. and Gen. Genet. 236: 369-373.

Brickell C. 1992. *The Royal Horticultural Society Encyclopedia of Gardening* (Print). London: Dorling Kindersley 356–357.

Budahn H, Peterka H, Mousa M, Ding Y, Zhang S, Li J. 2009. Molecular mapping in oil radish (*Raphanus sativus* L.) and QTL analysis of resistance against beet cyst nematode (Heterodera schachtii). *Th. and app. genet.*, 118: 775-782.

Cartea ME, Velasco P, Obregón S, Padilla G, de Haro A. 2008. Seasonal variation in glucosinolate content in Brassica oleracea crops grown in northwestern Spain. Phytoch. 2: 403-410.

Cho MA, Min SR, Ko SM, Liu JR, Choi PS. 2008. Agrobacterium-mediated genetic transformation of radish (*Raphanus sativus* L.). Plant Biot. *25*: 205-208.

Cox K, Beaton C. 2020. *Fruit and Vegetables for Scotland: What to Grow and How to Grow It*. Birlinn Ltd.

Crane MB and Mather, K. 1943. The natural cross-pollination of crop plants with particular reference to the radish. *Ann. of App. Biol.* 4: 301-308.

Curtis IS, Nam HG. 2001. Transgenic radish (*Raphanus sativus* L. longipinnatus Bailey) by floral-dip method–plant development and surfactant are important in optimizing transformation efficiency. *Transg. Res. 10*: 363-371.

Curtis IS. 2003. The noble radish: past, present and future. T. in Plant Sci. 8: 305-307.

Curtis IS. 2011. Genetic engineering of radish: current achievements and future goals. Pl. Cell Rep. 30: 733–744

Darmency H, Lefol E, Fleury A. 1998. Spontaneous hybridizations between oilseed rape and wild radish. Mol. Eco. *7*: 1467-1473.

Dixon, GR. 2007. *Vegetable brassicas and related crucifers* (No. 14). CABI.

Dongarwar LN, Kashiwar SR, Ghawade SM, Dongarwar UR. 2018. Varietal Performance of Radish (*Raphanus sativus* L.) Varieties in Black Soils of Vidharbha-Maharashtra, India. Int. J. Curr. Microbiol. App. Sci. 1: 491-501.

Eber F, Boucherie R, Broucqsault LM, Bouchet Y, Chèvre AM. 1998. Spontaneous hybridisation between vegetable crops and weeds. I. Garden radish (*Raphanus sativus* L.) and wild mustard (Sinapis arvensis L.). *Agronomie 18*: 489-497.

Ebert AW. 2013. Ex situ conservation of plant genetic resources of major vegetables. In Conservation of trop. plant speci. 373-417.

El-Ramady HR, Domokos-Szabolcsy É, Abdalla NA, Taha HS, Fári M, 2015. Postharvest management of fruits and vegetables storage. In *Sust. agricul. rev.* 65-152).

º

Elsayed AYA, Hamdino MI, Ahmed A, Shabana AI. 2016. Expected genetic gain in radish (*Raphanus sativus* var. red radicula) submitted to different procedures of selection. Egypt. J. Plant Breed. 20: 313 - 328, 2016

Fan L, Wang Y, Xu L, Tang M, Zhang X, Ying J, *et al*. 2020. A genome-wide association study uncovers a critical role of the RsPAP2 gene in red-skinned *Raphanus sativus* L. Hort. res. 7: 1-13.

Fita A, Rodríguez-Burruezo A, Boscaiu M, Prohens J, Vicente O. 2015. Breeding and domesticating crops adapted to drought and salinity: a new paradigm for increasing food production. Front. in Pl. Sci. *6*: 978.

Gamba M, Asllanaj E, Raguindin PF, Glisic M, Franco OH, Minder B, *et al*. 2021. Nutritional and phytochemical characterization of radish (*Raphanus sativus*): A systematic review. Trends in Food Sci. & Tech.

Gelvin SB. 2009. Agrobacterium in the genomics age. Plant Physiol. 150: 1665-1676.

Georgia AVS, Ockendon DJ, Gabrielson RL, Maguire. 1982. Self-incompatibility alleles in broccoli. *Horti. Sci. 17*: 748-749.

Giri A, Narasu ML. 2000. Transgenic hairy roots: recent trends and applications. *Biot. Adv. 18*: 1-22.

Giusti MM, Wrolstad RE. 1996. Characterization of red radish anthocyanins. *Journal of food science*, *61*: 322-326.

Gopalakrishnan TP. 2007. *Vegetable Crops*. New India Pub. 244–247.

Hashida T, Nakatsuji R, Budahn H, Schrader O, Peterka H, Fujimura T, *et al*. 2013. Construction of a chromosome-assigned, sequence-tagged linkage map for the radish, *Raphanus sativus* L. and QTL analysis of morphological traits. Breed. sci. 63: 218-226.

Hashida T, Nakatsuji R, Budahn H, Schrader O, Peterka H, Fujimura T, *et al*. 2013. Construction of a chromosome-assigned, sequence-tagged linkage map for the radish, *Raphanus sativus* L. and QTL analysis of morphological traits. Breed. sci. 63: 218-226.

Hayta S, Smedley MA, Demir SU, Blundell R, Hinchliffe A, Atkinson N, Harwood WA. 2019. An efficient and reproducible Agrobacterium-mediated transformation method for hexaploid wheat (*Triticum aestivum* L.). Plant Meth. 15: 1-15.

Hee-Ju Yu, Young-Min J, Jeong-Hwan M. 2017. Comparative analysis of the radish genome with Brassica genomes." In The Radish Gen. 53-69.

Hida K, Ashizawa M. 1985. Breeding of radishes for Fusarium resistance. JARQ. 3: 190-195.

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4885859

Hu T, Wei Q, Wang W, Hu H, Mao W, Zhu Q, *et al*. 2018. Genome-wide identification and characterization of CONSTANS-like gene family in radish (*Raphanus sativus*). PloS one, *13*: p.e0204137.

Jansen MD. 2021. An evaluation of tillage radish (*Raphanus sativus* L.) to alleviate post-construction soil compaction and germination potential under varying environmental conditions.

Jeong YM, Chung WH, Choi AY, Mun JH, Kim N, Yu HJ. 2016. The complete mitochondrial genome of cultivated radish WK10039 (*Raphanus sativus* L.). *Mitoch. DNA Part A*, *27*: 941-942.

Jeong YM, Chung WH, Mun JH, Kim N, Yu HJ. 2014. De novo assembly and characterization of the complete chloroplast genome of radish (*Raphanus sativus* L.). Gene. 551: 39-48.

Jin P, Zhu Z, Guo X, Chen F, Wu Y, Chen J, *et al*. 2020. Production and characterization of intergeneric hybrids by crossing radish with turnip and with Chinese kale. Euph, 216: 1-15.

Jing P, Zhao SJ, Ruan SY, Xie ZH, Dong Y, Yu LL. 2012. Anthocyanin and glucosinolate occurrences in the roots of Chinese red radish (*Raphanus sativus* L.), and their stability to heat and pH. *Food chem.* 4: 1569-1576.

Jones HA, Rosa JT. 1928. Truck Crop Plants. Macmillan & Co., Ltd., London. 538.

Kalia P. 2005. Root vegetable crops. J. of New Seeds, 2-3: 247-275

Kamei A, Tsuro M, Kubo N, Hayashi T, Wang N, Fujimura T, *et al*. 2010. QTL mapping of clubroot resistance in radish (*Raphanus sativus* L.). Theor. and App. Gen. 120: 1021-1027.

Kaneko Y, Bang SW. 2014. Interspecific and intergeneric hybridization and chromosomal engineering of Brassicaceae crops. Breed. sci. 64: 14-22.

Kaneko Y, Kimizuka-Takagi C, Bang SW, Matsuzawa Y. 2007. Radish. In *Vegetables*. 141-160).

Kaneko Y, Matsuzawa Y. 1993. Radish: *Raphanus sativus* L. In *Genetic Improvement of Vegetable Crops*. 487-510.

Kanjevac,. MM, Bojović BM, Todorović MS, Stanković MS. 2021. Effect of seed halopriming on improving salt tolerance in *Raphanus sativus* L. Krag. J. of Sci. 43: 87-98.

Kercher S, Conner JK. 1996. Patterns of genetic variability within and among populations of wild radish, Raphanus raphanistrum (Brassicaceae). Amer. J. of Bot. 11: 1416-1421.

Kim J, Manivannan A, Kim DS, Lee ES, Lee HE. 2019. Transcriptome sequencing assisted discovery and computational analysis of novel SNPs associated with flowering in *Raphanus sativus* in-bred lines for marker-assisted backcross breeding. *Hort. res.* 1: 1-12.

Kitashiba H, Li F, Hirakawa H, Kawanabe, T, Zou Z, Hasegawa Y, *et al*. 2014. Draft sequences of the radish (*Raphanus sativus* L.) genome. DNA res. 21: 481-490.

Kobayashi H, Shirasawa K, Fukino N, Hirakawa H, Akanuma T, Kitashiba H. 2020. Identification of genome-wide single-nucleotide polymorphisms among geographically diverse radish accessions. DNA Res.

º

Kremer JC. 1945. Influence of honeybee habits on radish seed yield. Q. Bull. Mich. Agric. Exp. Stn.

Kumar R, Solankey SS, Singh M. 2012. Breeding for drought tolerance in vegetables. *Veget. Sci. 39*: 1-15.

Kumar V, Trick M. 1993. Sequence complexity of the S receptor kinase gene family in Brassica. Mol. and Gen. Genet. 241: 440-446.

Lacroix B, Citovsky V. 2019. Pathways of DNA transfer to plants from Agrobacterium tumefaciens and related bacterial species. Annu. Rev. Phytopathol. 25: 231–251.

Lalonde BA, Nasrallah ME, Dwyer KG, Chen CH, Barlow B, Nasrallah JB. 1989. A highly conserved Brassica gene with homology to the S-locus-specific glycoprotein structural gene. The Plant Cell. 1: 249-258.

Lee O, Koo H, Yu JW, Park HY. 2021. Genotyping-by-Sequencing-Based Genome-Wide Association Studies of Fusarium Wilt Resistance in Radishes (*Raphanus sativus* L.). Gen. 12: 858.

Lee YJ, Mun JH, Jeong YM, Joo SH, Yu HJ. 2018. Assembly of a radish core collection for evaluation and preservation of genetic diversity. *Hort. Env. and Biot. 59*: 711-721.

Li F, Hasegawa Y, Saito M, Shirasawa S, Fukushima A, Ito T, *et al*. 2011. Extensive chromosome homoeology among Brassiceae species were revealed by comparative genetic mapping with high-density EST-based SNP markers in radish (*Raphanus sativus* L.). DNA res. 18: 401-411.

Li S, Zhang N, Li J, Xiang J. 2019. A new male sterile line of "Duanye-13" radish (*Raphanus sativus* L.) produced by ethyl methanesulfonate mutagenesis. Genet. Res. and Crop Evol. 66: 981-987.

Li Z, Zhou M, Zhang Z, Ren L, Du L, Zhang B, *et al*. 2011. Expression of a radish defensin in transgenic wheat confers increased resistance to Fusarium graminearum and Rhizoctonia cerealis. *Funct. & int. genom.* 11: 63-70.

Lim S, Lee EJ, Kim J. 2015. Decreased sulforaphene concentration and reduced myrosinase activity of radish (*Raphanus sativus* L.) root during cold storage. *Post. Biol. and Tech.* 100: 219-225.

Metz PL, Nap JP, Stiekema WJ. 1995. Hybridization of radish (*Raphanus sativus* L.) and oilseed rape (Brassica napus L.) through a flower-culture method. Euphy. 83: 159-168.

Mitsui Y, Michihiko S, Kenji K, Nobukazu N, Mari S, Misaki I, *et al*. 2015. The radish genome and comprehensive gene expression profile of tuberous root formation and development." Sci. rep. 5: 1-14.

Moghe GD, Hufnagel DE, Tang H, Xiao Y, Dworkin I, Town CD, *et al*. 2014. Consequences of whole-genome triplication as revealed by comparative genomic analyses of the wild radish Raphanus raphanistrum and three other Brassicaceae species. *The Plant Cell*, *26*: 1925-1937.

Muminović J, Merz A, Melchinger AE, Lübberstedt T. 2005. Genetic structure and diversity among radish varieties as inferred from AFLP and ISSR analyses. J. of the A. Soc. for Hort. Sci. 130*:* 79-87.

Nayik GA, Gull A. 2020. *Antioxidants in Vegetables and Nuts-Properties and Health Benefits*. *Sprin.*

Niikura S, Matsuura S. 2001. Genetic variation of the self-incompatibility alleles (S-alleles) in the cultivated radish (*Raphanus sativus* L.) by the PCR-RFLP method. Acta Horti. 546: 359–366.

Nishio T, Kusaba M, Sakamoto K, Ockendon DJ. 1997. Polymorphism of the kinase domain of the S-locus receptor kinase gene (SRK) in Brassica oleracea L. Theor. and app. Genet. 95: 335-342.

Nishio T, Sakamoto K. 2017. Polymorphism of self-incompatibility genes. The Radish Gen.. 177-188.

Nishio T. 2017. Economic and academic importance of radish. In The radish genome (pp. 1-10).

Nou S, Watanabe M, Isogai A, Hinata K. 1993. Comparison of S-alleles and S-glycoproteins between two wild populations of Brassica campestris in Turkey and Japan. Sex. Plant Rep. 6: 79-86.

Ockendon DJ. 2000, September. The S-allele collection of Brassica oleracea. In *III International Symposium on Brassicas and XII Crucifer Genetics Workshop* 539: 25-30).

Okamoto S, Sato Y, Sakamoto K, Nishio T. 2004. Distribution of similar self-incompatibility (S) haplotypes in different genera, Raphanus and Brassica. Sex. Plant Repr. 17: 33-39.

Patra JK, Das G, Paramithiotis S. Shin HS. 2016. Kimchi and other widely consumed traditional fermented foods of Korea: a review. Front. in microb. 1493.

Petropoulos SA, Sampaio SL, Di Gioia F, Tzortzakis N, Rouphael Y, Kyriacou MC, *et al*. 2019. Grown to be blue—Antioxidant properties and health effects of colored vegetables. Part I: Root vegetables. *Antioxidants*, 12: 617.

Porteus MH, Carroll D, 2005. Gene targeting using zinc finger nucleases. *Nature biot.* 8: 967-973.

Radchenko TG. 1966. Role of honeybees as pollinators in increasing the seed crop from cabbage and radish. Bdzhilnistvo.

Richharia R.H 1937. Cytological investigation of *Raphanus sativus*, Brassica oleracea, and their F 1 and F_2 hybrids. J. of Genet. 1:19-44.

Rissler J, Mellon M, Mellon MG. 1996. The ecological risks of engineered crops. MIT Press.

Rubatzky VE, Yamaguchi M. 1997. Cole Crops, Other Brassica, and Crucifer Vegetables. In *World Vegetables*: 371-417).

Ruffio-Châble V, Hervé Y, Dumas C, Gaude T. 1997. Distribution of S-haplotypes and its relationship with self-incompatibility in Brassica oleracea. Part 1. In inbred lines of cauliflower (B. oleracea var 'botrytis'). *Theo. App. Genet.* 94: 338-346.

Sakamoto K, Kushaba M, Nishio T. 1998. Polymorphism of the Slocus glycoprotein gene (SLG) and S-locus related gene (SLR1) in *Raphanus sativus* L. and self-incompatible ornamental plants in the Brassicaceae. Mol. and Gen. Genet. 258: 397–403.

º

Schreiner M, Huyskens-Keil S, Peters P, Schonhof I, Krumbein A, Widell S. 2002. Seasonal climate effects on root colour and compounds of red radish. J. of the Sci. of Food and Agricul. 11: 1325-1333.

Shen D, Sun H, Huang M, Zheng Y, Li X, Fei Z. 2013. RadishBase: a database for genomics and genetics of radish. Plant and Cell Physi. 54: 33.

Shirasawa K, Hirakawa H, Fukino N, Kitashiba H, Isobe S. 2020. Genome sequence and analysis of a Japanese radish (*Raphanus sativus*) cultivar named 'Sakurajima Daikon'possessing giant root. *DNA Res. 27*: 010.

Shrivastava P, Kumar R. 2015. Soil salinity: A serious environmental issue and plant growth promoting bacteria as one of the tools for its alleviation. Saudi j. of biol. Sci. 2: 123-131.

Simard MJ, Légère A. 2004. Synchrony of flowering between canola and wild radish (*Raphanus raphanistrum*). *Weed Sci.* 6: 905-912.

Singh A, Sharma S. 2020. Radish. In *Antioxidants in Vegetables and Nuts-Properties and Health Benefits*, 209-235.

Singh A, Sharma S. 2020. Radish. In *Antioxidants in Vegetables and Nuts-Properties and Health Benefits*. Spring. 209-235).

Singh S, Selvakumar R, Mangal M, Kalia P. 2020. Breeding and genomic investigations for quality and nutraceutical traits in vegetable crops-a review. *Indian J. of Horti.* 1: 1-40.

Snow AA, Palma PM. 1997. Commercialization of transgenic plants: potential ecological risks. Bio Sci. *47*: 86-96.

Sugimoto K. 2009. Evaluation of salt tolerance in Japanese wild radishes (*Raphanus sativus* f. raphanistroides Makino). Bull. of Minamik. Univ. *39*: 79-88.

Suzuki G, Watanabe M, Isogai A, Hinata K. 1997. Highly conserved 5′-flanking regions of two self-incompatibility genes, SLG9 and SRK9. Gene, 191: 123-126.

Tanaka Y, Tsuda M, Yasumoto K, Yamagishi H, Terachi T. 2012. A complete mitochondrial genome sequence of Ogura-type male-sterile cytoplasm and its comparative analysis with that of normal cytoplasm in radish (*Raphanus sativus* L.). *BMC genom.* 13: 1-12.

Tsuro M, Suwabe K, Kubo N, Matsumoto S, Hirai M. 2008. Mapping of QTLs controlling root shape and red pigmentation in radish, *Raphanus sativus* L. Breed. Sci. 58: 55-61.

Tsuro M, Suwabe,K, Kubo N, Matsumoto S, Hirai M. 2008. Mapping of QTLs controlling root shape and red pigmentation in radish, *Raphanus sativus* L. Breed. Sci. *58*: 55-61.

Tuberosa R, Salvi S. 2006. Genomics-based approaches to improve drought tolerance of crops. Trends in plant sci. 11: 405-412.

Tzfira T, Citovsky V. 2008. Agrobacterium: From Biology to Biotechnology. Spri.

Varshney RK, Bohra A, Yu J, Graner A, Zhang Q, Sorrells ME. 2021. Designing future crops: genomics-assisted breeding comes of age. Trends in Plant Sci.

Vishnu PS, Elavarasan R, Selvan T. 2020. Studies on radish salinity tolerance and their growth response analysis. Intern. J. of Adv. Res. in Bio. Sci. 7: 30-39.

Wang J, Qiu Y, Wang X, Yue Z, Yang X, Chen X, *et al*. 2017. Insights into the species-specific metabolic engineering of glucosinolates in radish (*Raphanus sativus* L.) based on comparative genomic analysis. *Scient. Rep.* 1: 1-9.

Wang Q, Zheng P, Zhang L. 2019. Identification and classification of S haplotypes in radish (*Raphanus sativus*). *Plant Breeding*, 138: 121-130.

Wang R, Mei Y, Xu L, Zhu X, Wang Y, Guo J, Liu L. 2018. Genome-wide characterization of differentially expressed genes provides insights into regulatory network of heat stress response in radish (*Raphanus sativus* L.). Funct. & int. genom. 18: 225-239.

Wang Y, Xu L, Chen Y, Shen H, Gong Y, Limera C, Liu L. 2013. Transcriptome profiling of radish (*Raphanus sativus L.*) root and identification of genes involved in response to lead (Pb) stress with next generation sequencing. PLoS One 6: 66539.

Xiaohui Z, Zhen Y, Shiyong M, Yang Q, Xinhua Y, Xiaohua C, *et al*. 2015. A de novo Genome of a Chinese Radish Cultivar. Hort. P. J. 1: 155-164.

Xu L, Wang L, Gong Y, Dai W, Wang Y, Zhu X, *et al*. 2012. Genetic linkage map construction and QTL mapping of cadmium accumulation in radish (*Raphanus sativus* L.). *Th. And App. Genet. 125*: 659-670.

Yarnell SH. 1956. Cytogenetics of the vegetable crops. II. Crucifers. *The Bot. Rev.* 2: 81-166.

Young HJ, Stanton ML. 1990. Influences of floral variation on pollen removal and seed production in wild radish. *Ecol.* 2: 536-547.

Yu D, Gu X, Zhang S, Dong S, Miao H, Gebretsadik K, *et al*. 2021. Molecular basis of heterosis and related breeding strategies reveal its importance in vegetable breeding. Hort. Res., 8: 1-17.

Yu X, Choi SR, Ramchiary N, Miao X, Lee SH, Sun HJ, *et al*. 2013. Comparative mapping of *Raphanus sativus* genome using Brassica markers and quantitative trait loci analysis for the Fusarium wilt resistance trait. Th. and app. Gen. 126: 2553-2562.

Zhan Z, Nwafor CC, Hou Z, Gong J, Zhu B, Jiang Y, *et al*. 2017. Cytological and morphological analysis of hybrids between Brassicoraphanus, and Brassica napus for introgression of clubroot resistant trait into Brassica napus L. PloS one, *12*: p.e0177470.

Zhang J, Qiu X, Tan Q, Xiao Q, Mei S. 2020. A comparative metabolomics study of flavonoids in radish with different skin and flesh colors (*Raphanus sativus* L.). *J. of Agricul. and Food Chem.* 49: 14463-14470.

Zhang J, Zhao J, Tan Q, Qiu X, Mei S. 2021. Comparative transcriptome analysis reveals key genes associated with pigmentation in radish (*Raphanus sativus* L.) skin and flesh. Scient. Report. 1: 1-11.

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 doi:10.20944/preprints202108.0514.v1

Zou Z, Ishida M, Li F, Kakizaki T, Suzuki S, Kitashiba H, *et al*. 2013. QTL analysis using SNP markers developed by next-generation sequencing for identification of candidate genes controlling 4-methylthio-3-butenyl glucosinolate contents in roots of radish, *Raphanus sativus* L. *PLoS One*. *8*: p.e53541.

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