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Improvement *oryza sativa* L.production using anther culture and molecular markers

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ABSTRACT

Rice plant is annual plant, belongs to genus Oryza, family Poaceae. In tropical areas, rice can survive as a perennial. It represents the staple food for half of the world's population, particularly poor people in less developed countries. The basic chromosome number of rice is n =12. The wild rice is distributed in humid tropics. There are more than 20 species of genus Oryza, but only two species, *Oryza sativa (*originated in the humid tropics of South and southeast Asia) and *Oryza glaberrima* (originated in Niger basin in Africa) are cultivated. Farmers favor traditional cultivars that early maturated in order to save land for other crop growth and to save water, scince rice needs more irrigation water than other grain crops. The difference in grain weight and quality within a panicle is variety-dependent, and also varies with the panicle type. Grains of aromatic rice (basmati rice) emit special aroma when cooked. Basmati rice is mostly grown in the traditional areas of North and northwestern part of Indian sub-continent for many centuries. It is the oldest common **progenitor** for most types. Basmati rice is photosensitive, requires relatively cooler temperatures to produce better aroma. Aroma is a complex mixture of volatile compounds, approximately 114 compounds and most of these aromatic compounds found in outer layers of grains. There is a controversy about the number of genes controlled aroma. Rice varieties improvement are represented in increasing the yield potential including modification of plant type, exploitation of heterosis, increasing yield stability, and increasing the yield potential under unfavorable environments. The importance of biotechnology in rice improvement is highlighted and some of the techniques used, such as somaclonal variation, embryo rescue, somatic hybridization, anther culture, molecular markers in rice breeding and introduction of novel genes into rice, are outlined.

INTRODUCTION

Rice plant belongs to genus *Oryza*, family *Poaceae*, order *Poales*[1]. The basic chromosome number of rice is n =12. Rice is the second largest cereal crop, represents the staple food for half of the world's population[2] and is the main food for poor people in less developed countries[3]. Rice plant is unique among economic crops due to its varietal forms and adaptation to diversity of soils and climates[4]. It includes both annual and perennial species.

CROP DISTRIBUTION

The wild rice is distributed in humid tropics throughout the world. Morphologically, the materials of wild rice were divided into Asian, African, American and Oceanian types[5]. The term "wild rice" can refer to the wild species of *Oryza*, but conventionally refers to species of the related genus *Zizania*, both wild and domesticated. Rice is annual plant, but in tropical areas it can survive as a perennial and can produce a ratoon crop and survive for up to 20 years[6].

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There are more than 20 species of rice (*Oryza* genus) but only two species, *Oryza sativa*, which originated in the humid tropics of south and south-east Asia. And *Oryza glaberrima* originated in Niger basin in Africa, are cultivated. *Oryza sativa* is grown world wide due to its better yield and adaptability to local growing conditions.

ORRGIN OF DISTRIBUTION OF RICE

It is believed that the cultivated rice *Oryza glaberrima* Steud developed from *Oryza breviligulata* Chev. and *Oryza sativa* originated from *Oryza perennis* Moench. In 1926, Vavilov[7] considered that large number of crop species including rice originated from Hindustan center which including India, Assam and Burma (which runs from East to West of Himalays in old continent and also, mountainous regions which runs from South to North of Andes in the new continent). Chowdhury and Ghose[8] discovered in excavations in Hasthinpura in northern India that rice already existed by 1000 B. C. and in ruins of Yangshao and China by 2600 B. C. The rough rice was discovered in 1973 in Hemudu (is a village near Ningpo in central China), this rice dates back to 6.000 to 7.000 years old according to measurement using C14. *Oryza glaberrima* Steud is only grown in central part of West Africa. In 1956, Porteres stated that African rice originated around 1500 B. C. and the primary center of origin of African rice might have been located in swampy areas of upper stream of Niger River. But, the secondary center might have been in south western part of Guinea coast, which is estimated to be established 5.000 years behind the primary center[9-12].

According to Zohary and Hopf[13], *O. sativa* was introduced to the Middle East in Hellenistic times, was familiar to both Greek and Roman writers. They reported that a large sample of rice grains was recovered from a grave at Susa in Iran, dated to the first century after date. Rice was grown in the Po valley in Italy, although, Pliny wrote that rice was grown only in Egypt, Syria, Cilicia, Asia Minor and Greece[14-17].

CROPYIELD

Rice yield per unit of land in Egypt is the highest in the world due to excellent climatic conditions of the country, soil types, adequate irrigation water and comprehensive research and extension programs for developing and introducing new high-yielding varieties as well as improved cultural practices. Rice is one of the most important cereal crops in Egypt, which cultivated on about 1.5 million Fadden area, and became one of the most profitable field crops for Egyptian farmers especially after liberalization of agriculture and cancellation of the rice quota delivery policy. Rice is considered one of the potential export crops that can provide foreign exchange required for economic development in Egypt. Rice productivity in Egypt became the highest in the world especially during the last few years. According to Agriculture commodity council of Egypt in 2003; Egypt produced 6 million tones of rice in that year, which represent 1 % of total international rice production and nearly 35 % of total Africa rice production. The net return of rice export for Egypt was nearly 190 million dollars in 2003. Another important socioeconomic impact of rice cultivation is the large number of labor force employed in the rice sector. According to El-Deeb[18], cultivation 1 hectare needs about 124 labor-days, meaning that more than 73 million labor-days are required annually during the summer season for rice cultivation. Moreover, a large number of workers are involved in rice milling, marketing and trading activities[19-22].

Farmers favor traditional cultivars that early maturated in order to save land for other crop growth. Moreover, rice needs more irrigation water than other grain crops[23-26]. So, breeding genotypes early to maturation will save more water. Fifteen days earlier to maturation for rice crop save 10-15% of irrigation water which equals 1.4×10^9 m^3/year[27]. The great differences in flowering time of rice plant result in a wide variation in grain development, and weight and quality within a panicle[28-30].

Egyptian consumers favor short grain which give high milling out-turn (> 70 %)[31-34]. The difference in grain weight and quality within a panicle is variety-dependent[35], and also varies with the panicle type[30, 36]. Commonly, the varieties with big- or/and erect panicle have greater variation in grain weight and quality within a panicle than those with small- or/and curve panicle[37-39]. In recent two decades,

many compact-panicle rice varieties characterized by short panicle and high grain density within a panicle were released and intensively planted in the southeast China[30]. These compact panicle varieties are commonly characterized by high yield potential comparing to loose panicle ones[40-43], and lower in filled grain percentage and grain weight[30, 36]. The variation in grain development and quality within a panicle was attributed to the difference in the activity of the enzymes relevant to starch synthesis[44-47].

AROMATIC RICE

Aromatic rice is a kind of rice, its grains emits special aroma when cooked[48]. The aromatic compounds found in all plant parts except root[49-50]. The aromatic compounds are a complex mixture originates from a small amount of volatile compounds, nearly 114 compounds[51-54]. Basmati rice is aromatic rice variety which characterized by extra long, superfine, and slender grains having a length to breadth ratio of more than 3.5, sweet taste, soft texture, delicate curvature and extra elongation with least breadth-wise swelling on cooking and less glutinous[55]. Basmati rice is mostly grown in the traditional areas of North and northwestern part of Indian sub-continent for many centuries. It has been grown in the foothills of the Himalayas for thousands of years. In India, it is produced on either side of Indus valley. Basmati rice is the oldest common progenitor for most types. Basmati is the king of all varieties of rice, nourishes for the body tissues, is easy to be digested, and elongates almost twice (70-120 % over the pre-cooked grain) upon cooking but does not fatten much. So, it considered India's gift to the whole world. Basmati rice transforms rice dishes into extraordinary meals because it is fluffy rice and its grains stay separate when cooked. Using spices, nuts, dried fruits, vegetables and herbs made basmati rice dishes very delicious meals. Yet throughout history, it has been on royal menus and in various cultures was used as the main dish from the pilaf of Turkey, polou of Persia, pilafs of the Steppes, the isotos and paellas of the Mediterranean, to the pulau of India which has been served to great sultans, maharajahs, shahs and emperors.

Basmati rice is excellent nutritional source, provides human with 20 % or more of recommended daily nutritional value. As the analysis of 200 gm of cooked basmati rice grain variety, found it releases 205 k calories which were found in 7.4 g of protein, 44.5 g of carbohydrates, 0.44 g of fats and 0.63 g of fiber. Moreover, it enriched also with 1.9 mg iron, 11.8 µg selenium, 0.26 mg thiamine and 2.3 mg niacin. It is a good food for people who are at high risk for diabetes, prostate cancer or heart disease. Because, it contains selenium which is helpful for those who are at high risk for prostate cancer or heart disease[56]. And it contains higher amount of fiber, which slows digestion of food and prevents insulin spikes[57-62]. Moreover, its grains double in length when cooked, which in turn increase the feel with satiety in comparison with normal rice. This is likely to decrease the risk of type 2 diabetes.

Scented rices are preferred by the consumers all over the world due to its flavor, palatability and nutritional value[63]. They are characterized by extra long slender grain with soft and fluffy texture of cooked rice, all these characters made them highly priced in domestic and international markets[64-69] and high export value in the international market. Aromatic rice varieties enjoy special attention in the international market because they are highly in demand in South and southeast Asia and Middle East countries. Recently, the changes in Egyptian national economic policy and the increase in influx of tourists into the country have raised the need for more aromatic rice. Focus of Egyptian rice breeding program, therefore, is on the selection and breeding of a new rice variety possessing high-yielding ability and aromatic trait with a good grain quality.

Basmati rice is photosensitive, requires relatively cooler temperatures (25 °C in day and 21 °C at night) to produce better aroma. The proper ageing is also required to obtain the correct aroma and flavor of basmati rice, to reduce its moisture content. The aroma is affected by temperature during ripping and storage[70]. Lipid content and fatty acid composition were affected by cultivar differences in heading time and cropping period. This variation mainly caused by temperature during filling period of the grain[71-75]. As aroma result from degradation of fatty acid, it has been found that under temperature between 20-22 °C produces equal amount linoleic and

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oleic acid. The increase of temperature above this level increases oleic acid content. On the contrary, the decrease of temperature below this level increases linoleic acid[76], which in turn increases aroma amount. So, aromatic rice needs to grow under low temperature. On the other hand, low temperature appeared to provide higher concentration of aromatic compound 2-acetyle-1-proline[77-81]. The high temperature during storage found to decrease 2-AP content and increase fat acidity of rice[82], and after short time rice aroma become stale.

Basmati rice plant is also affected by environmental condition and plant nutrition[83]; osmotic stress (drought) and nitrogen fertilizer which would increase L-proline content during ripening[84-69] and timely sowing and transplanting[85-88]. Time of sowing and transplanting is an important factor in determining grain yield and quality parameters, early sowing or planting date produces higher yields and prolonged the duration of vegetative phase resulting in a tall and leafy crop. Such a crop is more prone to lodging because of excessive vegetative growth and plant height.

Aroma is a complex mixture of volatile compounds[89], approximately 114 compounds[51] and most of these aromatic compounds found in outer layers of grains[89], where lipid is found. So, there were a close relationship between aroma and fatty acids[90]. The lipid deposited in aleuron layer is hydrolyzed by lipase enzyme to glycerol and fatty acids. Then, the desaturated fatty acids as linoleic acid and linolenic acid are peroxidized by lipoxygenase enzyme then finally are converted to volatile compounds (aldhyde and keton compounds and sulfur compounds) which are responsible for the aroma of rice after harvest. Lipoxygenase isozyme was detected in rice seeds[91-95], and in rice bran and embryo[96-97]. 2–Acetyl-1-proline (2-AP) is believed to be the major component to produce aroma[50]. 2-Acetyl-1-proline is the reason of consumer's acceptance to aromatic rice and hexanal is off flavor. The ratio of 2-acetyl-1-proline and hexanal differ according to variety[98]. The nitrogen source of 2-AP was L-proline, while, the carbon source was not the carboxyl group of L-proline; was acetyl group[99]. So, when proline is accumulated in plant under various types of stress, It would be positively correlated with 2-AP formation[100-103].

There is a controversy over the number of genes controlling aroma trait in rice. The number of genes controlled aroma could be a single recessive gene[104-105] or single dominant gene[106], two dominant genes[107], three or four complementary recessive genes[108], a single recessive gene interacting with an inhibitor gene[109]. Dong *et al.*[110] indicated that aroma was controlled by a single recessive gene located on chromosome 8. However, Pinson[111] stated that aroma is controlled by a single recessive gene or two recessive genes which varied with varieties. In indica rice landrace, a dominant aroma gene was identified on chromosome 11[112]. In addition, Siddiq *et al.*[113] reported that two recessive genes controlled aroma in indica variety was located on chromosomes 5 and 9. On the other hand, Chen *et al.*[51] made a study using indica and japonica varieties and reported that aroma is controlled by a single recessive gene on chromosome 8 and placed the *fgr* locus between RM8264 and RM3459 with a physical distance of nearly 69 kbp.

CROP IMPROVEMENT

Introduction

Rice varieties improvement are represented in increasing the yield potential including modification of plant type, exploitation of heterosis, increasing yield stability, and increasing the yield potential under unfavorable environments. The importance of biotechnology in rice improvement is highlighted and some of the techniques used, such as somaclonal variation, embryo rescue, somatic hybridization, anther culture, molecular markers in rice breeding and introduction of novel genes into rice, are outlined[114]. Anther culture could be used for rapid production of transgene-homozygous lines from transgenic rice plants, even from transgenic plants with multi-locus insertion[115].

Tissue culture

Tissue culture is the branch of science in which growth and development of cells and tissues occurred *in vitro* under controlled conditions. The cell has the totipotency to grow and regenerate to complete plant. Tissue culture has contributed to the release

of new rice varieties. It will enable plant breeders to achieve results more quickly and efficiently and will help them to attain breeding goals not feasible using conventional techniques. The tissue culture does not replace conventional breeding. It is a complement to other breeding methods[116].

First tissue culture for rice was from immature embryo[117], and a node[118]. Heller's inorganic salts supplemented with glycine (3 ppm), D, L-tryptophan (60 ppm), nicotinic acid (0.5 ppm), pyridoxine (0.5 ppm), thiamin (0.5 ppm), 2,4–D (2, 4– dicholoro phenoxy acetic acid) (2 ppm), yeast extract (0.5 %), sucrose (2 %) and (0.6 %) agar were used for callus induction. The effect of N.A.A. (naphthalene acetic acid) and 2,4–D on callus induction from seed was studied by Maeda[119]. In 1967, he observed cultivar differences in callus induction. Nishi *et al.*[120] obtained the first regenerated rice plant. Futsuhara[121] cultured rice root using indica and japonica cultivars, and their hybrids. He observed that all japonica cultivars well regenerated while indica and its hybrids completely failed[122].

Anther culture

The anther culture is employed to obtain double haploid lines (DH) from crosses for different plant breeding objectives. Prior to develop haploid rice, haploid plants were produced in *Datura innoxia* and Tobacco. Niizeki and Oono[123] made first successful callus induction of haploid from rice through anther culture. Since then, the anther culture technique has been refined greatly. It is now possible to produce haploids from the anther culture of many japonica and indica rices, although the frequency of plant regeneration is lower in indica varieties. A number of varieties and improved breeding lines have been developed through anther culture in China, Korea, Japan and the United States. Most of the anther culture derived varieties are japonica. Indica rices are generally regarded as recalcitrant for anther culture[124].

Doubling the chromosome in which the haploid material is complemented; is a rapid tool for inducing homozygosity, which in turn shortens the time required for the development of new rice varieties. Because, the development of homozygous lines using conventional breeding requires five to seven generations, homozygosity can be achieved within two generations using anther culture combined with spontaneous or chemically induced chromosome doubling. The segregation of the progeny of such crosses is complex and selection in earlier generations inefficient. But with anther culture, the selection becomes easier. The fertility of japonica/indica hybrids is improved by anther culture as demonstrated by Li *et al.*[125] and Enriquez *et al.*[126]. When the seed set of f_1 hybrids was 26–36 % fertile, the back crossing improved this rate to 45–50 %, whereas anther culture derived H_1 lines from the same crosses exhibited as high as 80 % fertility[116]. Moreover, anther culture not conceal the phenotypic expression of the recessive genes[127] and aroma is controlled by a single recessive gene[101-103] which in turn will increase the ratio of the produced aromatic lines. New lines with gametoclonal variation were produced due to the medium hormones.

Uni-nucleate pollens, the developing stage of pollens is effective in culture[128]. The highest induction ratio was obtained when middle uni-nucleate pollens were used for tissue culture[129]. These pollens can be identified when the length between auricles of flag leaf and penultimate leaf is between 5–7 cm and the colour of glumaceous flower is light green.

As one of the growth conditions of donor plant, temperature is the most critical effect in frequency of callus induction and regeneration. Field temperature between 18 ~ 20 ºC induced highly differentiated calli than those cultivated in 26 ~ 28 ºC.

The response of genotypes for culturing ability is ordered as from high to low as follows; glutinous rice, japonica, japonica/indica hybrids, hybrids of indica, indica and non glutinous rice[122]. The alanine content in anthers affects induction and regeneration process. This was very pronounced in the anthers of Indica type which contains alanine lower than other types. Callus production in this type was promoted by addition of alanine to culture medium[128].

The pretreatment (panicles kept in refrigerator at 10 ºC for 10 days) is believed to cause microspores to converge into uni-nucleate stage and this is due to respiration reduction for microspores and reduction for consumption of substances. Moreover, it lengthens the viability of anthers and pro-

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vides favorable conditions for the maintenance of pollen grains. Zhou *et al.*[129] reported that the time required for pre-treatment is as reduced as temperature goes down. In japonica type, the most effective temperature was 5 ºC for 7 days in comparison with 10 ºC for 10 days and 13 ºC for 10-14 days. While for indica type, there were different suitable temperature 3–5 ºC for 10 days, 6-8 ºC for 10–15 days and 9–10 ºC for 15–20 days.

Anther culture derived lines in rice and their parents under saline and non-saline soil conditions were evaluated. Data were collected on grain yield, yield components, and agronomic characters. The results indicated that the possibility of regenerating recombinants with desirable characters such as good plant type, salinity tolerance, higher yield and resistance to pests and diseases from both the parents are high[130]. Fifteen doubled haploid salt tolerant rice lines were developed via anther culture. Two sensitive lines were crossed with a salt tolerant line to transfer its salt tolerant character to lines of DH[131]. Fertile double-haploid of interspecific (japonica x japonica, japonica x indica and *Oryza sativa* x *O. glaberrima* crosses) progeny were generated through anther culture[132]. Rice lines resistant to salt stress were obtained by in vitro selection of anther-derived callus[133]. Twenty five of double haploid lines were produced through anther culture, were grown with their respective parents for field evaluation for blast reaction as well as agronomic and yield characters. Three lines of these lines produced high yield comparable to their parents and exhibited resistance to blast[134]. Eight anther culture derived lines regenerated from two single crosses were evaluated. These lines were selected for their excellent behavior in upland culture condition and their good grain quality. These lines were blast resistant, and scored days to flowering ranged from 86 to 98, plant height ranged from 100 to 130 cm and the grain yield ranged from 2.0 to 4.9 ton/ha[135]. A new rice variety, namely "Marianna" was developed via anther culture of F_1 plants of the cross Belozem x Plodiv 22. This variety has better characteristic features than both the parents. It has growth duration period of 121-125 days, plant height of 120 cm and a 1000-grain weight of 32.5 g, even yield were higher than the check (5.4 t/ha)[136]. A restorer line TG8 was developed through anther culture. This restorer exhibited normal fertility with both Indica and Japonica CMS lines. It has also short stature desirable plant type, high tillering ability, large panicle, good grain quality and resistance to rice blast disease. On the other hand TG8 possessed a dominant widely compatible gene (WCG)[137]. Double haploid lines derived through anther culture were evaluated. They were produced from 12 indica rice varieties, six basmati cultivars and 31 F_1/F_2 heterotic hybrids. Several of these lines had a greater number of productive tillers and greater panicle length. Some of these lines had a desirable character and selected for further evaluation[138]. Parage 401 rice variety was developed through anther culture. It was produced from the F_1 Prabhavati x Basmati 370. It was evaluated for yield related characters in multi location experiments in four years and superior lines were selected. AC parag 401 selections showed superior quality characters and it was released as parag 401 for cultivation in India[139].

Application of anther culture to crosses of indica rice has been limited. Even if with one japonica parent, due to recalcitrant nature of indica rice varieties which made poor callus proliferation and high percentage of albino plants which are currently recognized as the major problem in indica rice varieties[140]. The question is how to activate these recalcitrant genes?. The answer is that media requirements must be optimized for androgensis and green plants regeneration[141-142]. Hartke and Lorz[143] tested 15 indica rice lines and found seven of them produced embryogenic calli, only four regenerated to plants. The rate of success in indica rice anther culture can be enhanced by improving the composition of tissue culture medium especially by manipulating plant growth regulators[144-145] and osmotic pressure[146], elements composition[147], and using maltose as a carbon source[148]. Basmati rice varieties have proven recalcitrant to anther culture[149]. Several media Heh5[150], RZ[147] and Potato dextrose agar media differed in macro and micro elements composition, maltose or sucrose and presence or absence of casein, had been used as suitable medium for indica type. Indica hybrids showed increased anther response in medium (Sk-1) which had increased NO_3^- level and containing casein and with-

out ammonium salts. Although, Basmatic rice x indica rice hybrids on RZ medium without casein showed superior induction and regeneration[151]. Inorganic salts modifications did not show any significant difference except nitrogen with indica rice hybrids. The addition of total nitrogen and the combined use of levels of nitrate and ammonium affected anther response and regenerability of green plants. When ammonium or nitrate used in medium as the lonely source, the response of anther was poor[147]. This is like the studies with rice somatic tissues[152] in which ammonium or nitrate as the sole nitrogen source failed to support growth. Twenty media modification for three basic media (N6, SK-1 and RZM) used. They modified with different combinations of KNO_3 (31-34 mgm) and $(NH_4)_2SO_4$ (2-2.5 mgm) and casein hydrolysate. KNO_3:$(NH_4)_2SO_4$ ratio was 90:10, was superior for callus induction and plant regeneration in indica type. On the contrary, KNO_3:$(NH_4)_2SO_4$ ratio was 10:90, was optimal for japonica types. The medium devoid from casein and ammonium salts was very poor. When N6 medium had only half the amount of ammonium which showed significantly superior anther response and plant regeneration efficiency. In comparison with medium containing casein instead ammonium salt[147]. NO_3^-:NH_4^- ratio changed the sensitivity of rice somatic calli to 2, 4-D in callusing medium[152]. Indica type anthers were found to contain lower amount of alanine than japonica type. When alanine added to medium, callus production was promoted[129]. The analysis of chemical constituents of Basmatic rice showed that Basmatic rice contains higher amount of fiber, iron, selenium, thiamine and niacin and double amount of protein [87, 153, 154]. RZM medium was found to be more suitable than other media. It scored higher number of anther forming calli (1-10 calli) in comparison (1-2 calli) in N6 modified medium and anther respond quicker (3 weeks) in comparison other media (4-5 weeks)[155]. Maltose was beneficial for callus induction from microspores[92]. Using maltose instead of sucrose has been found to be superior in several anther culture studies and is widely in use[92]. RZM mediun with sucrose, obtained lower callus induction frequencies than RZM medium with maltose (2.6-77.9 %)[100]. Sucrose is believed to double osmolarity of the medium, because it rapidly hydrolyzed to glucose and fructose[100]. Production of albino plants has been the major problem in rice anther culture, especially in indica rice varieties and the hybrids involving indica rice parents[91-92]. Green to albino plant regeneration ratios from anther culture calli of indica x Basmati rice hybrids can be improved by transferring calli to regeneration medium at a younger stage, use of lower incubation temperature than 26 ^oC and improving induction and regeneration media[100]. Efforts have been made to optimize media requirements for androgenesis and green plant regeneration in indica rice[100].

As these lines are aromatic varieties, it was lucky to make modifications with proline, yeast or both. Suprasarma *et al*.[153] found that L-proline supplementation could yield an increase in aroma production in cell cultures of basmati rice; Yoshihashi[96] confirmed the role of proline as a precursor in the aroma formation in var. Khao Dawk Mali 105. Kishor *et al*.[154] stated that proline effects on embryogenesis and plant regeneration in recalcitrant rice callus. Raval and Chattoo[155]) stated that proline not only increased callus growth but also showed an increase in embryogenic callus formation. Schieberle[156] reported that ornithine found in baker's yeast, was a principal precursor of 2- AP during baking whereas L-proline led to formation of 2-AP by *B. cereus*[157]. The amino acids related to proline and glutamate inter conversion pathway (i.e., Pro, Orn and Glu) were used as probable precursors of 2-AP. Gly, Met, Trp and His, showed an increase in 2-AP concentration when Orn and Glu were added to solution. But, when Pro was added to the solution, it increased the concentration by more than three folds. While, addition of Gly, Met, Trp and His did not increase 2-AP content in rice leaves. Moreover, yeast contains free fatty acids as linoleic acids[158]. Linoleic acid, its further degradation causes aroma. For all these reasons, medium modified with proline or/and yeast was used to increase aroma in the plants regenerated through anther culture or give a better response. The low concentration (200 mgm) of yeast gave better response[159].

Molecular DNA markers

Plant breeders and geneticists widely studied and

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employed the linkage between morphological markers and economically important traits, which in most cases are quantitative in nature, strongly influenced by environment and expensive to evaluate directly[160]. Plant breeders used a simple morphological character as a marker for the quantitative trait. The advent of molecular DNA markers has revolutionized the genetic analysis of crop plants and provided not only geneticists, but also physiologists, agronomists and breeders with valuable new tools to identify traits of importance[161]. The advent of molecular technique opened the ways for a new and unlimited number of markers which covering the whole genome. These markers are inert and not affected by environmental fluctuations. Molecular markers such as RAPDs, AFLPs and SSRs are simple, rapid and accurate[162]. They were used very efficiently to pick out the tomato plants that are tolerant to stress[163] and to select drought resistant genotypes in common bean[164]. Molecular markers can also be used to trace genetic or epigenetic changes at the genome level[165-166].

Microsatellites or simple sequence repeats (SSRs), are DNA sequences with repeat lengths of a few base pairs and variation in the number of nucleotide repeats, can be detected with PCR by selecting the conserved DNA sequences flanking the SSR as primers. SSR markers were developed for rice in 1993[167]. 500 Microsatellite markers are well-distributed and has been genetically mapped into the rice genome which links the genetic and physical maps with the genomic sequence of rice, facilitating studies that seek to determine the relationship between the structure and function of genes and genomes[168]. SSR markers are co-dominant, can detect high level of allelic diversity, highly repeatable, and identify a single locus and targets hypervaiable regions of the genome[169]. SSR markers are useful not only to characterize the relationship between heterosis and marker genotype heterozygosity, but also to identify chromosome segments that may have significant effects on yield and its component traits in rice[94]. The application of molecular markers has great impact on improving the efficiency of rice breeding program and has the capacity to assist in the selection traits that are expensive or laborious to assess[170]. Moreover, the molecular marker can be evaluated from a single seedling leaf or seed samples, allowing selection to occur before expression of the trait. A quality character such as aroma is very difficult to assess accurately. However, using molecular markers in preliminary screening would reduce labour cost and assist in selection, by avoiding non-fragrant or heterozygous individuals from sensory or chemical assessment of aroma.

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