

Maintenance Breeding of Pusa Basmati
Varieties

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Abstract

Genetic gain, achieved by any breeding and varietal development programme, can be realized only when there is a robust seed production system, underpinned by a systematic maintenance breeding programme. Breed (variety) and seed are two facets of the same coin—one without another is irrelevant. The full potential impact of a new improved variety, however excellent it may be, cannot be realized, unless it is supported by a strong seed multiplication, distribution and marketing system. The popularity and complete dominance of the ICAR-IARI bred Basmati varieties is evident from the increase in percent share of breeder seed indents of Pusa Basmati varieties in the total breeder seed indents of all Basmati varieties. The details of maintenance breeding and the success of Pusa Basmati varieties are described in this chapter.

Keywords

Basmati · Genetic gain · Mechanical mixtures · Outcrossing · Breeder seed production

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12.1 Introduction

India has achieved significant productivity gains in major cereals post-green revolution (post-GR) era of 1984–2017. The annual gain in rice productivity in different phases after green revolution has been computed as 68% and 117% in 1984–2000 and 2001–2017, respectively (Fig. 12.1) (Yadav et al. [2019](#page-24-0)). Similarly, impressive genetic gains have also been made in quality and productivity of Basmati varieties, a speciality group of rice varieties, having great aroma and premium culinary attributes (Singh et al. [2018a](#page-24-0)). This progress has largely been attributed to development and adoption of improved cultivars and crop management technologies. Significant efforts go into breeding programmes and development of trait-specific high-yielding varieties with inbuilt resistance to pests and diseases, besides tolerance to various biotic and abiotic stresses and quality attributes. Seeds are the carriers of these genetic gains to farmers and ultimately to the consumers. Quality seed alone contributes a 15–20% increase in productivity. ICAR-Indian Agricultural Research Institute (ICAR-IARI) bred Basmati varieties (commonly known as 'Pusa Basmati' varieties) have made a significant impact in converting these genetic gains into economic gains for different stakeholders associated with Basmati rice production, processing and export. Cumulative foreign exchange earnings of a single landmark Basmati variety, Pusa Basmati 1121, in one decade (2008–2017) have been to the tune of US \$20.8 billion (Singh et al. [2018a](#page-24-0)). Pusa Basmati varieties (Pusa Basmati 1121, Pusa Basmati 1509, Pusa Basmati 1, etc.) are dominating the seed chain (Figs. [12.2](#page-2-0) and [12.3](#page-2-0)).

Basmati rices possess unique organoleptic properties (aroma and taste) due to which these are very popular in the international markets. However, cultivation of

Fig. 12.1 Rice productivity (kg ha^{-1}) trend since 1950 (Yadav et al. [2019](#page-24-0))

Fig. 12.2 Cumulative breeder seed indent of Basmati varieties during the last 10 years, 2010–2019 (Breeder Seed Allocation Plans [2020](#page-23-0))

Fig. 12.3 Percentage share of breeder seed indents of Pusa Basmati varieties in total breeder seed indents of all Basmati varieties (2010–2019)

Basmati is geographically limited to North-Western India and Punjab in Pakistan. In India, Basmati production is confined only to seven states (Punjab, Haryana, Himachal Pradesh, Delhi, Uttarakhand, Jammu and Kathua districts of Jammu and Kashmir and 27 districts of Western Uttar Pradesh). This specific region has been earmarked as Geographical Indication (GI) for Basmati rices in 2016 by

Geographical Indication Registry, Government of India (GI No. 145, Certificate No. 238 dt. 15.02.2016). Typically, GI conveys an assurance of quality and distinctiveness which is essentially attributable to the fact of its origin in that defined geographical locality, region or country (Department of Promotion of Industry and Internal Trade [2021\)](#page-23-0).

Basmati—being one of the most popular speciality rices in the EU and the Middle East—attracts premium prices and is subject to stringent tests to differentiate between authentic Basmati from non-Basmati rices (Nader et al. [2019\)](#page-23-0). The authenticity definitions by the UK Code of Practice on Basmati Rice (CoP) of [2017](#page-23-0) clearly stipulate that when the description of the product is 'Basmati rice', the non-Basmati rice content must not exceed 7%. This tolerance is in place to take account of seed impurity and other segregation issues at origin ([http://www.riceassociation.](http://www.riceassociation.org.uk) [org.uk\)](http://www.riceassociation.org.uk). This clearly underscores the critical need for maintaining the supply of highquality genetically pure seed for the production of Basmati rice.

Traditional Basmati varieties are tall, lodging and disease prone, photoperiod sensitive and poor yielders. Pusa Basmati 1, the world's first evolved, semi-dwarf, photoperiod-insensitive, high-yielding Basmati variety developed by ICAR-IARI, New Delhi, in 1989, brought a paradigm shift in Basmati breeding (combining Basmati quality with higher yield and semi-dwarf stature). ICAR-IARI continues to play the flagship role in Basmati breeding with the development of several popular Basmati varieties like Pusa Basmati 1509, Pusa Basmati 6, Pusa Basmati 1637, Pusa Basmati 1718 and the recently released Basmati variety Pusa Basmati 1692 (notified in 2020).

12.2 Maintenance Breeding

Maintenance breeding (used synonymously with varietal maintenance) is a basic technique which primarily deals with the purification and maintenance of varieties. Notwithstanding its simplicity, this process has a profound impact on the spread and enhanced productive life of a variety. 'Variety maintenance' is the perpetuation of a small stock of nucleus seed, as the basis of all future multiplication and production of a variety, either by repeated multiplication of a small stock by a precise procedure, controlling the relationships of the plants in the stock, or by storage (Laverack [1994\)](#page-23-0).

12.2.1 Why Maintenance Breeding?

'Thou shall not sow thy fields with mingled seed', Leviticus 19:19. Thus were the Hebrews enjoined to sow their fields with unmixed seed. The early farmers were aware of the fact that varieties of crops deteriorate with time unless directed efforts are made towards maintaining the integrity of the variety (Kadam [1942](#page-23-0)). Plant breeding is often described as 'plant evolution' directed by man. One of the principal constraints of the conventional breeding methods is that selection decisions about the merits of diverse lines are largely based on their phenotypes. Genotype describes the allelic constitution of an individual at one or more loci, while phenotype is the observable expression of one or more traits (Singh and Singh [2017](#page-24-0)). The various traits of an organism can be grouped into two categories: (1) qualitative traits, governed by one or few major genes or oligogenes, each of which produces a large effect on the trait phenotype, and (2) quantitative traits, governed by several genes, each having a small individual effect on trait phenotype, which are usually cumulative. Most of the traits of biological and economic importance are quantitative or metric traits. The phenotypic expression of quantitative traits is significantly influenced by the environment and, often, an interaction between genotype and environment.

The phenotype can be expressed by the following equation:

$$
P = \mu + G + E + (G \times E)
$$

where P is the phenotype of a quantitative trait (governed by multiple genes), μ is the population mean, G is the effect of genotype of the concerned individual, E is the effect of the environment on the expression of the trait and $(G \times E)$ is the interaction component. A precise estimation of G, E and G \times E components of phenotypic variation for different quantitative traits is one of the continuing quests for plant breeding (Singh and Singh [2017](#page-24-0); Singh [2012](#page-23-0)). This is also a challenge in maintenance breeding as the production of different classes of seeds including nucleus and breeder seed is essentially done based on the phenotype.

A comprehensive study on the importance of maintenance breeding in the first miracle rice variety IR 8 provided strong justification for continuous maintenance breeding to counter rapidly evolving biotic and abiotic stresses. Maintenance breeding plays a decisive role in the adaptation of newly developed varieties to changing environmental conditions, which have a deleterious impact on older varieties (Peng et al. [2010](#page-23-0)).

12.2.2 Varietal Deterioration and Maintenance Breeding

Two essential characteristics of a cultivar are (1) identity and (2) reproducibility. In self-fertilizing crops, a cultivar generated from a single, homozygous genotype will be uniform in appearance, whereas a cultivar increased from a mixture of genotypes will exhibit a range of genetic variability according to that present in the mixture. This is assuming that the plant originally selected is homozygous at all loci—an assumption plant breeders often make, but this assumption is seldom met (Sleper and Poehlman [2006](#page-24-0)). There may be several minor loci still segregating even in the F_{10} generation leading to the production of off-types in a large population. Kadam [\(1942](#page-23-0)) in his classical paper on varietal deterioration enumerated seven critical factors responsible for the degradation of varieties over time.

12.2.2.1 Developmental Variations

Seed crops which are grown under different environments over consecutive generations may exhibit differential growth responses leading to the production of such variation. In order to minimize these variations, it is advisable to restrict the seed production of the varieties in their areas of adaptation.

12.2.2.2 Mechanical Mixtures

Mechanical mixtures are one of the most important reasons for varietal deterioration and are mainly attributed to human negligence (Fig. 12.4). These are the leading causes for litigation between seed producers and farmers. Shattering of grains in rice (an important source of mixture) results in volunteer plants (self-sown plants). Thus, care should be taken that the land used for seed production is free from volunteer plants. This stipulation is a mandatory protocol for seed production as per Indian Minimum Seed Certification Standards (IMSCS [2013\)](#page-23-0). Proximity of threshing floors, unclean tarpaulins or the use of the same contaminated seed drills, seed bins and gunny bags and mistakes in handling seed during seed processing are the main reasons for mechanical mixtures. These can be avoided by taking utmost care during every step of seed production and processing.

Fig. 12.4 Mechanical mixtures: major factor for varietal deterioration (result of human negligence)

12.2.2.3 Mutations

Mutation is a sudden heritable change in the genotype of an organism. The organisms with such heritable changes are known as mutants. Mutations are of two types (spontaneous and induced) depending upon their origin. A spontaneous mutation is one that occurs in nature, while an induced mutation results from a mutagenic agent. What appears to be a spontaneous mutation may have been induced, because all plants in nature are subjected to low dosages of natural radiation (Sleper and Poehlman [2006\)](#page-24-0). In nature, plant mutation rates occur between 10^{-5} and 10^{-8} during adaptation and evolutionary processes. These frequencies of natural or spontaneous mutations are extremely low (Zhon-hua et al. [2014](#page-24-0)). Mutations per se do not pose a serious threat in seed production and varietal maintenance of Basmati rices as well.

12.2.2.4 Natural Outcrossing

The extent of natural outcrossing in rice varies from 0% to 3% , depending on the cultivar and the environment, with an average of about 0.5% (Sleper and Poehlman [2006\)](#page-24-0). Sometimes there is lag between spikelet opening and bursting of the anther resulting in outcrossing. Though rare, but outcrossing in rice is not an exception (Fig. [12.5a](#page-7-0), b). In a study on outcrossing (OC) in winter wheat, Martin [\(1990](#page-23-0)) observed that there is no question that OC occurs during the multiplication stages of cultivar development. It is likely that OC is most serious when experimental lines are growing side by side in early-generation plant or head rows and, subsequently, in initial small increase plots. If the incidence of outcrossed seed could be reduced in seed replanted from such nurseries, the production cost of all classes of certified seed could be reduced significantly by decreasing the amount of roguing required to meet purity standards for the cultivar.

An interesting observation has been reported by Kadam ([1942\)](#page-23-0) that farmers in Konkan generally believe that mixtures suddenly appear in the third year of growing an improved variety. This is due to the fact that in that year natural F_1 plants have segregated for various characters. Perpetuation of such plants, in addition to mechanical mixtures, increases the proportion of dominant types, until in the end a variety resembles a conglomeration of various types. The natural outcrossing can be with off-types, diseased or undesirable plants.

Bateman [\(1947](#page-23-0)) in an exhaustive work on contamination of seed crops reported that there are two types of contamination of seed crops: (1) mechanical mixtures and (2) cross-pollination between varieties (admixture of foreign seed at harvesting or admixture of foreign pollen at flowering). The degree of genetic contamination in seed fields due to natural crossing depends upon four variables: (a) the breeding system of species, (b) isolation distance, (c) varietal mass and (d) pollinating agent. Contamination generally decreases, as the isolation distance between the varieties is increased; however, there still may be miniscule traces of contamination over wide distances. Appropriate isolation of seed crops per se is, therefore, a primary prerequisite for the seed production of crop plants cross-pollinated by winds or insects.

Fig. 12.5 (a) Outcrossed plants in rice seed field of Pusa Sugandh 5. (b) Outcrossed plant with pigmented apiculi in rice seed field of Pusa Sugandh 5

12.2.2.5 Minor Genetic Variations

Varieties appearing phenotypically uniform and homogeneous at the time of their release may still have minor genetic variations. This could be due to the fact that in a typical breeding programme, the seed multiplication is done after its identification/ release. Till the identification/release of a variety, the genotypes are grown in smaller

area, and when grown in larger area for seed multiplication, it makes it possible to identify the off-types in a large population of plants. Some of these minor genetic variations may be lost during subsequent production cycles due to selective elimination by the environment (Kadam [1942](#page-23-0); Agrawal [1991\)](#page-23-0). Remnant of these genetic variants can be eliminated to a large extent by careful nucleus and breeder seed production.

12.2.2.6 Selective Influence of Diseases

The selective influence of diseases is also an important factor in varietal deterioration. New varieties often become vulnerable to new races of diseases caused by obligate parasites and are often out of seed production programmes (Kadam [1942;](#page-23-0) Agrawal [1991](#page-23-0)). With the current focus on incorporating disease resistance in Basmati varieties, a number of disease resistance varieties have been bred by ICAR-IARI through molecular marker-assisted backcross breeding (MABB) (Singh et al. [2019](#page-24-0))—for example, Pusa Basmati 1718 (a MAS-derived bacterial blight (BB) resistant Basmati rice variety possessing two genes, $xal3$ and $Xa2I$) (Singh et al. [2018b\)](#page-24-0), Pusa Basmati 1637 (a MAS-derived near isogenic line of Pusa Basmati 1 possessing Pi9 gene for blast resistance) (Singh et al. [2017a](#page-24-0)) and Pusa Basmati 1728 (a MAS-derived near isogenic line of Pusa Basmati 6 carrying two genes for BB resistance, viz. $xal3$ and $Xa21$) (Singh et al. [2017b\)](#page-24-0). Taking up systematic seed production programme of these varieties having inherent resistance to specific diseases, the selective influence of these specific diseases can be reduced or eliminated.

12.2.2.7 Segregation Due to Residual Heterozygosity in the Cultivars

Premature release of varieties, still segregating for resistance and susceptibility to diseases or other factors, is also an important factor of varietal deterioration. In addition, heritable variations on account of recombination and polyploidization may occur in varieties during seed production (Kadam [1942](#page-23-0); Agrawal [1991](#page-23-0)). Avoiding hasty release of varieties and putting in seed chain only the stabilized varieties can reduce residual segregation or stability issues to a large extent.

It is apparent from the aforesaid discussion that genetic variation may appear within a seed lot due to multiple reasons, viz. mechanical contamination, undesirable pollination, residual segregation, recombination and mutations. These diverse factors ensure that no variety is likely to retain the precise allele frequencies established by breeder without continuous monitoring (Laverack and Turner [1995\)](#page-23-0). The process of continuous intervention to monitor and maintain the genetic purity of the variety is termed as maintenance breeding. It is usually the fag end of varietal development and the first step in the initiation of seed production. Figure [12.6](#page-9-0) depicts an analogy adapted from 0 to 1 (Thiel and Masters [2014\)](#page-24-0). The value 0 to 1 indicates an innovation (variety) and 1 to n is scaling up (seed production). Maintenance breeding is at the cusp of breeding and seed production. In practice, most of the issues pertaining to quality, adaptability and yields are encountered at initial scaling up of the variety and should be sorted out at this stage. Pusa Basmati

1692, a new variety notified in 2020 (Singh et al. [2020](#page-24-0)), is now at the cusp of maintenance breeding and its large-scale seed production.

12.3 Basmati Varieties Maintenance Breeding and Breeder Seed Production

At present, 34 varieties have been notified as Basmati varieties till 15 July 2021 (Notified Basmati Varieties [2021\)](#page-23-0) (Table [12.1\)](#page-10-0). To understand the varietal dynamics and consumer preferences, a comparison of breeder seed indents of all Basmati varieties in seed chain in the last decade (2010–2019) has been made in Figs. [12.2](#page-2-0) and [12.3.](#page-2-0) A total of 24 Basmati varieties figured in breeder seed indents in the last decade. Two varieties Basmati 217 and Mahi Sugandha have zero breeder seed indent, and three varieties have almost negligible cumulative breeder seed indent of less than 0.50q each ((Type 3 (0.10q), Ranbir Basmati (0.37q), Punjab Basmati 4 (0.32q)) during this decade. Top three peaks were occupied by Pusa Basmati varieties viz., Pusa Basmati 1121, Pusa Basmati 1509 and Pusa Basmati 1 (Fig. [12.2](#page-2-0)). This complete dominance of Pusa Basmati varieties gives an idea about demand pull of these varieties, implicitly indicating that varieties per se are not only excellent, but also gives an insight about IARI's ability to consistently saturate the seed markets with genetically pure high-quality seeds which is produced from nucleus seed (product of maintenance breeding).

All the IARI (Pusa) bred Basmati varieties undergo systematic maintenance breeding at ICAR-IARI, Regional Station, Karnal (dedicated to maintenance breeding and seed production). A brief description of some important Basmati varieties (Pusa Basmati 1121, Pusa Basmati 1509, Pusa Basmati 6, Pusa Basmati 1718) along with specific comments on the maintenance breeding and breeder seed production is discussed in following sections.

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12.3.1 Pusa Basmati 1

Pusa Basmati 1 was released for commercial cultivation in 1989 (Fig. 12.7). It is the first evolved Basmati variety having semi-dwarf stature, high yield potential and photoperiod insensitivity. Development of this variety by ICAR-IARI was a turning point in Basmati breeding in India. Pusa Basmati 1 is a product of cross between Pusa 150 and Karnal Local. It combines unique traits from diverse lineage. Pusa 150 is a breeding line derived through a convergent breeding approach involving many high-yielding non-aromatic rice varieties (Taichung Native 1, IR 8, IR 22, etc.) and traditional Basmati rice variety (Basmati 370) used as quality trait donor. Karnal Local was a selection from the traditional Basmati rice collection, Haryana Basmati Collection 19 (HBC 19) from the Karnal district of Haryana (with better grain and cooking quality), which was later released as Taraori Basmati in 1996 (Singh et al. [2004\)](#page-24-0). Pusa Basmati 1 is still in demand as regular breeder seed indents of this variety are received till date. It is being maintained at ICAR-IARI, Regional Station, Karnal, since 1989 (notification year). Maintenance breeding comment: kind of variants observed in nucleus/breeder seed plots: (1) awn less off-types; (2) flowering variants.

Fig. 12.7 Pusa Basmati 1: outstanding example of maintenance breeding (notification year 1989)

12.3.2 Pusa Basmati 1121

Pusa Basmati 1121 is an exquisite Basmati rice variety with exceptional grain and cooking quality notified for commercial cultivation in 2005 and subsequently (after the amendment) for the states of Delhi, Punjab and Haryana in 2008 (Table [12.1\)](#page-10-0). The superior linear cooked kernel elongation of this unique variety was derived from parents Basmati 370 and Type 3 (used as donors for grain and cooking quality traits). Accumulation of favourable loci for extra-long grain and exceptionally high linear cooked kernel elongation was possible through transgressive segregation, due to selective inter-mating of the sister lines showing better linear kernel elongation in the segregating generations. As many as 13 rice varieties/enhanced germplasm (including Basmati 370 and Type 3) were used to bring together the favourable alleles at multiple loci for agronomic, grain and cooking quality characteristics in the development of Pusa Basmati 1121 (Fig. 12.8) (Singh et al. [2018a\)](#page-24-0).

Modern varieties being the products of complex lineage and multiple crosses, consequently the varietal maintenance of these varieties also becomes quite arduous. Different types of variants do crop up in these varieties during repeated cycles of seed production. Many times it becomes very difficult to keep the exact combination of the favourable alleles brought together by breeder, almost intact in repeated cycles

Fig. 12.8 Pusa Basmati 1121 pedigree showing contribution of several varieties. Years in parentheses indicate the year in which crossing was initiated (1966) and the year of release of variety (2003) (Singh et al. [2018a](#page-24-0))

of multiplication. Pusa Basmati 1121 is a typical example of a difficult variety to maintain due to its complex ancestry (Fig. [12.8](#page-12-0)) various types of variants have been observed (Fig. [12.9a, b\)](#page-14-0). Consistent and recurrent cycles of varietal maintenance, nucleus and breeder seed production (Fig. [12.10\)](#page-15-0) have enabled this variety to make a significant contribution in farmer prosperity. Maintenance breeding comment: challenging variety to maintain. Kind of variants observed in nucleus/breeder seed plots: (1) tall off-types; (2) dwarf off-types; (3) grain size off-types; (4) awned off-types.

12.3.3 Pusa Basmati 1509

Pusa Basmati 1509 is a very popular Basmati rice variety notified in 2013 for commercial cultivation. It has semi-dwarf plant stature (a plant height of 95–100 cm), sturdy stem and non-lodging and non-shattering habit as compared to Pusa Basmati 1121. It is suitable for multiple cropping systems with a seed-to-seed maturity of around 115–120. It has an average yield of 4.1 t/ha with potential yield as high as up to 7.0 t/ha under good management conditions. Pusa Basmati 1509 possesses aromatic extra-long slender grains (8.41 mm) and good kernel length after cooking (19.1 mm) (Singh et al. 2014). Its area is fast increasing, and this variety has significant export potential (Fig. [12.11](#page-15-0)). Maintenance breeding comment: kind of variants observed in nucleus/breeder seed plots: (1) tall off-types; (2) grain size off-types.

12.3.4 Pusa Basmati 6

Pusa Basmati 6 is very popular in niches of southern Punjab and north-western districts of Haryana. The major chunk of breeder seed of this variety is used in these districts (Fig. [12.12](#page-16-0)). Pusa Basmati 6 is also popularly known as Pusa 1401. It has semi-dwarf plant stature with sturdy stem. This variety has a kernel that retains uniform shape after cooking, as against kernel shape of Pusa Basmati 1121 (tapering end after cooking). Pusa Basmati 6 possesses strong aroma along with minimum chalkiness (\lt 4%) (Singh et al. [2018a](#page-24-0)). Maintenance breeding comment: kind of variants observed in nucleus/breeder seed plots: (1) grain size off-types; (2) tall off-types; (3) dwarf off-types.

12.3.5 Pusa Basmati 1718

Pusa Basmati 1718 is a product of marker-assisted backcross breeding having two genes (xa13 and Xa21) governing bacterial blight (BB) resistance. It is a MAS-derived near isogenic line of popular variety Pusa Basmati 1121. Pusa Basmati 1718 has been released for Basmati-growing states of Haryana, Punjab and Delhi. With a seed-to-seed maturity of 136–138 days, it has an average productivity of 4.6 t/ ha (maximum yield potential 6.0 t/ha) (Singh et al. [2018b](#page-24-0)). It possesses long slender **Fig. 12.9** (a) and (b) Pusa Basmati 1121 maintenance and purification. Variants in a paired row, raised from single true-to-type panicle

Fig. 12.10 Journey of Pusa Basmati 1121. An established brand. Breeder seed indent and production from 2005 onwards

Fig. 12.11 Journey of Pusa Basmati 1509: a brand in making. Breeder seed indents and production from 2013 (notification year) onwards

grains (8.1 mm) and very good kernel length after cooking (17.0 mm). It also has very less grain chalkiness, intermediate amylose content (22.2%) and strong aroma (Singh et al. [2018a\)](#page-24-0). This variety is becoming quite popular and in the near future is likely to occupy significant area under Basmati cultivation. Maintenance breeding comments: (1) tall off-types; (2) grain size variants.

Fig. 12.12 Journey of Pusa Basmati 6: breeder seed indents and production from 2010 (notification year) onwards

12.4 Basmati Varieties Maintenance Breeding Procedure

The procedure adopted is panicle to row method:

- Single 'true-to-type' panicles (350–500 in number) are selected.
- Selected 'true-to-type' panicles are threshed individually. Each threshed panicle is critically screened for seed characteristics.
- For Basmati varieties, a small portion of seed of each panicle is subjected to grain and cooking quality testing.
- Seeds of panicles not conforming to seed characteristics or not performing well in cooking quality are rejected.
- In the case of varieties developed through MABB, an additional step is undertaken. Seedlings are raised from part of the seed from each panicle used for cooking, for screening the presence of target alleles of the genes incorporated using either gene-based or gene-linked markers (Fig. [12.13\)](#page-17-0). Any panicle having any inadvertent plant without possessing the R-allele of the disease resistance genes is summarily rejected.
- Seeds of remaining 200–250 panicles are raised in panicle rows. A slight modification is raising of paired rows from a single panicle. Raising of paired rows from single panicle helps in better comparison amongst the selected panicles (Figs. [12.14a](#page-18-0), b).

Fig. 12.13 Amplification profile of the molecular analysis of panicles of Pusa Basmati 1718 for the presence of BB resistance genes, $xal3$ and $Xa21$, using gene-based marker, $xal3$ prom and $pTA248$, respectively. All the panicles amplified 500 bp fragment for $xal3$ prom and 950 bp with $pTA248$ corresponding to the resistance alleles of xa13 and Xa21. Hence, all these panicles which were possessing desirable grain and cooking quality are only considered for raising panicle to row progenies for nucleus seed production. M, 100 bp ladder; P_1 , Pusa Basmati 1121 (susceptible check); and P_2 , Improved Pusa Basmati 1 (resistant check); 1–22, panicles harvested from nucleus seed plot for further maintenance, nucleus and breeder seed production

- Generally, the row length is kept 5 m long. Spacing between the rows (30 cm) and between two paired rows (60 cm). The plant-to-plant spacing is kept 20 cm. Thus, a 5 m long paired row raised from a single panicle would be having 50 plants (i.e. 25 plants/row and 50 plants/paired row). The wider spacing of 60 cm between two paired rows helps in the proper expression of individual plants and critical observation and screening of different paired rows. This layout can be modified as per availability of seedlings per panicle for transplanting for each paired row.
- A thorough screening of panicle rows at different crop growth stages is done. Diagnostic characteristics based on DUS guidelines (PPV&FRA [2007](#page-23-0)) for conduct of test for distinctiveness, uniformity and stability on rice are very useful in screening.
- Panicle rows not conforming to 'true-to-type' plant type or showing off-types are totally discarded as and when observed.
- Remaining selected panicle rows are harvested and threshed individually. And again harvested and threshed seed of each individual panicle row is critically examined.
- Finally, seeds of all retained 'true-to-type' panicle rows are bulked to get genetically pure high-quality nucleus seed.

Integration of cooking and grain quality test and screening for disease resistance genes in the maintenance breeding programme itself has significantly enhanced the consumer preference of these varieties. Maintenance breeding plots of specific varieties are raised in such a way that these are surrounded by the breeder seed plots of the same variety. This simple intervention (nucleus seed plots surrounded by breeder seed plots) not only prevents outcrossing with undesirable pollen as breeder seed plots act as buffer but also helps in visual comparison providing both micro and macro views of the variety (Fig. [12.14a, b\)](#page-18-0).

Fig. 12.14 (a) Pusa Basmati 1121 nucleus seed production (panicle paired rows). (b) Pusa Basmati 1121 maintenance breeding plots (nucleus seed) surrounded by breeder seed plots (to prevent outcrossing)

12.5 Maintenance Breeding, Seed Production, Off-Types and Rogues

Adequate understanding of terminology, namely maintenance breeding, generation system of seed multiplication, seed chain, off-types and rogues, helps in conceiving and executing a proper seed production programme. There are three recognized classes of seed in the Indian generation system of seed multiplication (i.e. breeder, foundation and certified seed), and seed supply chain follows a three- to four-tier system of multiplication (Breeder seed \rightarrow Foundation seed \rightarrow Foundation/Certified seed \rightarrow Certified seed). The seed multiplication cycle starts with 'breeder seed', which is dependent upon availability of high-quality nucleus seed (a product of varietal maintenance). Any issue with the genetic purity of the breeder seed lot (presence of contaminants/off-types/mixtures) gets multiplied and results in an exponential increase in these contaminants in succeeding generations. The presence of these contaminants may lead to loss of identity and requisite traits of the variety for which it has been specifically bred.

12.5.1 Off-Types

Off-types are defined as plants showing a distinct phenotype from the sown variety and are unknown as a variety (Lee et al. [2013\)](#page-23-0). The proportion of off-types in any particular population or seed lot depends on four factors: (a) rate of addition of plants in each generation and the number of generations of multiplication, (b) proportion of progeny of off-types which are also off-types (i.e. the stability of these off-types in subsequent multiplications), (c) relative rate of multiplication of off-type plants and (d) effectiveness of removal of off-types by roguing at each generation (Laverack and Turner [1995\)](#page-23-0). Bateman ([1946\)](#page-23-0) described contamination as 'obvious' and 'cryptic' in the deterioration of certain British vegetable seed stocks when isolation distances were inadequate. He classified off-types produced by cross-pollination with other varieties into 'obvious' types, where a distinct phenotype was produced, and 'cryptic' where the resulting variant genotype was not easily seen from phenotypic characters. Obvious off-types would be seen more easily and removed. Cryptic off-types would be more difficult to detect and so could spread in the population with potentially serious consequences for yield and quality. This description of obvious and cryptic contamination is very aptly delineated by the famous optical illusion (Fig. [12.15](#page-20-0)) titled 'The Young Girl—Old Woman' (Attneave [1971](#page-23-0)). The cryptic off-types are generally camouflaged in larger seed production plots. These can only be removed effectively in maintenance breeding plots, where limited numbers of panicle rows are being critically observed, as against larger seed production plots (Figs. [12.16](#page-20-0) and [12.17](#page-21-0)).

Fig. 12.15 Obvious or cryptic. The optical illusion 'The Young Girl—Old Woman' (the young woman's chin is also the old woman's nose). (Adapted from Attneave [1971\)](#page-23-0). Cryptic off-type plants are often camouflaged

Fig. 12.16 Obvious (tall off-type)

Fig. 12.17 Cryptic (grain size off-type)

12.5.2 Roguing

Roguing may be defined as the selective removal of undesirable plants from a seed crop on the basis of visual inspection in the field in order to improve one or more quality attributes of the seed lot to be harvested. Roguing for genetic purity is an attempt to maintain the original genetic base of a variety. By defining the limits of phenotypic variation and removing non-conforming plants, it seeks to achieve uniformity expected or required. Roguing represents a continuation of the maintenance process in order to restrict variation within an acceptable level, but it is necessarily imprecise because judgments about genotype (G) are made from the phenotype, which is the result of genotype and environment ($G \times E$) interaction (Laverack and Turner [1995](#page-23-0)). Proper understanding of rice plant morphology and its descriptive features helps in undertaking effective roguing. DUS guidelines of Rice gives the descriptors of rice plant (62 characteristics; 29 asterisk characteristics) (PPV & FRA [2007\)](#page-23-0). These guidelines are very helpful in identifying off-types from true-to-type plants in maintenance breeding plots as well as for undertaking roguing operations in large-scale seed production plots (Figs. [12.16](#page-20-0) and 12.17).

The point to be understood here is that in maintenance breeding plots (nucleus seed plots), we never undertake roguing. It is the summary rejection of panicle rows expressing any sort of variants. Roguing operations are undertaken only in largescale seed production plots (breeder, foundation, certified or truthfully labelled seed).

12.6 Conclusions

Putting together all the disparate components discussed above (maintenance breeding, seed chain, seed production, true-to-type, off-type and roguing), it can be concluded that varietal development, maintenance breeding and seed production represent a continuum. For any effective crop varietal development programme, maintenance breeding plays a pivotal role in its ability to saturate the area under cultivation with genetically pure seed. Basmati export trade in international markets is going to have a bigger and wider footprint in the coming years. If this sector is to be developed, akin to software industry with a potential to generate more than Rs. 50,000 crores forex in next 3–5 years (Basmati Rice-Life in Science with Pallava Bagla [2020\)](#page-23-0), then the Basmati rice exports have to be tailor-made to meet consumers' preferences. Global Basmati markets are now becoming mature with very discerning buyers in the EU, North America and the Middle East, where authentic Basmati rice gets a premium price. Nader et al. [\(2019](#page-23-0)) clearly depict the kind of scrutiny and pedigree checks these markets undertake for authenticity testing of this premium Basmati grain (Fig. 12.18). Another interesting and insightful indicator is UK Code of Practice on Basmati Rice (CoP) of [2017,](#page-23-0) which clearly

Fig. 12.18 EU strict authenticity checks: pedigree of Basmati rice varieties based on information about their breeding history, which was available in the public domain. (Adapted from Nader et al. [2019\)](#page-23-0)

stipulates that when the description of the product is 'Basmati rice', the non-Basmati rice content must not exceed 7%. This tolerance is in place to take account of seed impurity and other segregation issues at origin ([http://www.riceassociation.org.uk/](http://www.riceassociation.org.uk/content/1/47/2017-basmati-code-of-practice.html) [content/1/47/2017-basmati-code-of-practice.html](http://www.riceassociation.org.uk/content/1/47/2017-basmati-code-of-practice.html)). This type of rigorous scrutiny of Basmati rice is an explicit imperative for a very strong varietal maintenance programme for all the Basmati varieties in seed chain, to keep India's dominance in Basmati markets.

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