International Journal of Statistics and Applied Mathematics 2023; SP-8(5): 1139-1145

International Journal of Statistics and Applied Mathematics

ISSN: 2456-1452
Maths 2023; SP-8(5): 1139-1145

https://www.mathsjournal.com
Received: 10-07-2023
Accepted: 05-08-2023

Poonam Nawalkar
Department of Plant Molecular Biology and Biotechnology, College of Agriculture, IGKV, Raipur, Chhattisgarh, India

Sunil Kumar Verma
Department of Plant Molecular Biology and Biotechnology, College of Agriculture, IGKV, Raipur, Chhattisgarh, India

Corresponding Author:
Poonam Nawalkar
Department of Plant Molecular Biology and Biotechnology, College of Agriculture, IGKV, Raipur, Chhattisgarh, India

Polyploidy breeding in forage crops: A review

Poonam Nawalkar and Sunil Kumar Verma

Abstract
Indian farming is heavily reliant on crops and livestock because the majority of our population relies on them for a living. Our population is always growing (it may reach 1400 million by 2025), and the animal population is growing as well. As the animal population grows, so does the demand for fodder during the peak months of the year. Because livestock is heavily dependent on the crop wastes. It is necessary to breed variations which reveals that milk and meat w production will expand 2.8 and 3.3 percent every year, respectively, while the cattle population, which is now about 500 million, will rise at a rate of 1.23 percent per year. Livestock production accounts for 7% of India's national GDP (source: employment and sustenance for 70% of rural population). The Crop Improvement Division was founded in 1967 with the purpose of improving the forage production and nutritional properties of diverse farmed and pasture species from various agro-climatic areas in India by using alien and indigenous genetic resources via breeding and selection. Fodder crop enhancement necessitates a multidisciplinary integrated strategy to cultivating high-yielding cultivars, as well as the creation of materials and information through strategic and fundamental research. Cultivated cereal/grain crops, annual legumes, and perennial grasses (cultivated/rainfed), and range legumes are the main fodder crops.

Polyploidy is a major evolutionary force in both wild and cultivated plants. Polyploid organisms are typically more vigorous and, in certain situations, outperform their diploid counterparts in a number of ways. Over the last century, many plant breeders have focused on the extraordinary superiority of polyploids, inducing polyploidy and/or using natural polyploids in a variety of methods to develop ever-improving plant cultivars. The "Gigas" effect, buffering of harmful mutations, enhanced heterozygosity, and heterosis (hybrid vigour) are some of the most important impacts of polyploidy for plant breeding. In terms of tools, cultivars with higher production levels, improved product quality, and increased resistance to both biotic and abiotic stressors have been developed. Green fodder is vital for animal health, which ultimately decides animal output in terms of milk and meat. To meet the projected demand, we must boost production, utilise untapped feed resources, increase land area (not for human use), and imports. To fulfil expected demand, the green forage supply must expand at a 3.2% annual rate. Because of ongoing pressure to cultivate commercial and human-use crops, the gap between demand and supply of fodder is widening. The only way to close the demand-supply imbalance is through genetic enhancement of fodder crops. Before beginning any breeding plan with forage crops, it is critical to understand their breeding behaviour and constraints, as they differ from other crops.

When crossing between two species is impossible owing to ploidy level discrepancies, polyploids can operate as a bridge for gene transfer. Furthermore, polyploidy often results in lower fertility due to meiotic mistakes, permitting the development of seedless cultivars. Wheat is the greatest example of a naturally polyploid plant. Triticale was the first man-made polyploidy, generated by mixing tetraploid wheat with diploid rye. Triticale is presently grown in 37 countries, generating 17.1 million tonnes in total. Auto polyploids play a vital role in fruit growth, particularly seedless fruit formation, which triploid polyploids such as Watermelon, Guava, and Grape create. Polyploidy has a huge impact on production. Polyploidy has a substantial influence on agricultural yield, including strawberries, cabbage, chrysanthemums, and roses. Every year, the gift of polyploidy cotton and sugarcane provides us with a high productivity.

Keywords: Watermelon, Guava, Grape create

Introduction
When an item or individual contains more than two basic or monoploid sets of chromosomes, this is referred to as polyploidy. Polyploidy is ubiquitous in nature and serves as an important mechanism for adaptation and speciation. Polyploidy has occurred in at least half of the evolutionary history of known angiosperm species.

Polyploidy results in the "gigas" effect, or gigantism, which promotes cell size expansion and, as a result, bigger plant organs.

Improvement of Forage Crops and Varietal Development

Over the previous 59 years, a nationwide network has introduced over 300 fodder crop types such as berseem, lucerne, cowpea, guar, field bean, oats, pearl millet, Sehima, Chrysopogon, Cenchrus, Dinanath grass, Guinea grass and others. IGFRI has supplied 48 high-yielding forage cultivars with increased quality and stress tolerance that are appropriate for the country's diverse climatic zones. The vast majority of perennial fodder grasses are polyploid and reproduce through apomixis. The vast majority of perennial fodder grasses are polyploid and reproduce through apomixis. To aid breeding, rare sexual lines of Buffel grass (Cenchrus ciliaris) and Guinea grass (*Panicum maximum*) have been found.

Furthermore, apomixis, perenniality, and multicut nature were passed from wild crop relatives to bajra, and a variety (BBSH-1) appropriate for dry and semi-arid locations under rainfed circumstances was produced. The new discovery of viable and seed-producing BN hybrids will overcome the constraints of bulky rooted slip multiplication and transport. Micropropagation techniques were improved in numerous forage grasses and legumes, and a transformation approach for genetically altering Lucerne for weevil resistance was developed. Furthermore, the Institute has developed pest and disease control strategies for main fodder crops (cultural/biological/botanicals/chemicals/integrated IPM).

The presence of more than two sets of chromosomes is referred to as polyploidy. To comprehend polyploidy, a few fundamental terms must be defined. The letter "x" represents the fundamental set of chromosomes, whereas the letter "2n" represents the total number of chromosomes in a somatic cell. Acquaah *et al.* (2007) [1] discovered that the number of chromosomes in a somatic cell is twice that of gametes. The vast majority of nucleated animals are diploid (2n), with two sets of chromosomes, one from each parent. Despite the fact that most eukaryotes have diploid somatic cells, meiosis allows them to make haploid gametes.

Polyploidy is defined as having more than two full sets of chromosomes per cell nucleus. It is common in nature and plays an important role in adaptation and speciation. Polyploidy occurs when a typically diploid cell or organism acquires one or more additional sets of chromosomes.

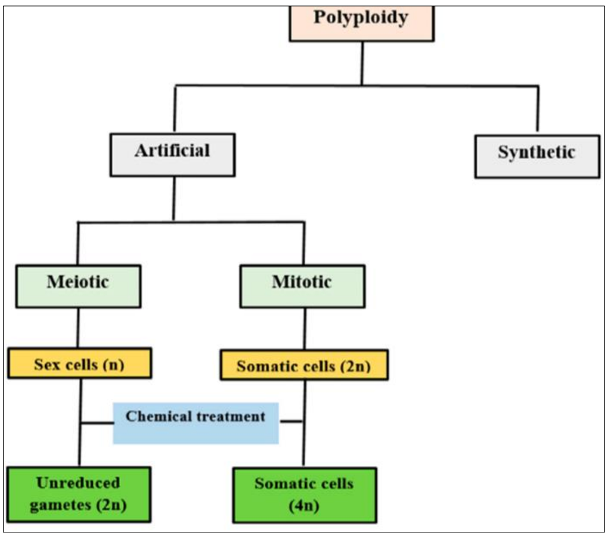

Fig 1: Protocol for induction of polyploidy

Polyploidy in plants can be produced by exposing certain plant parts to colchicine, such as plant tissue or cells. Colchicine is an alkaloid that functions as an antimitotic metabolite. It is often employed in plant cells to promote polyploidy and chromosomal doubling.

Polyploidy can occur spontaneously by a number of processes, including meiotic or mitotic failures and the fusing of unreduced (2n) gametes (Comai *et al*., 2005) [12]. In both wild and cultivated plant species, autopolyploids (such as potato) and allopolyploids (such as canola, wheat, and cotton) are observed. Polyploidy is a common approach employed in plant breeding to overcome a hybrid species' infertility. Triticle is a cross between wheat and rye (*Triticum turgidum* x *Secale cereale*).

It combines the best qualities of both parents, yet the first hybrids are infertile. The hybrid becomes viable after polyploidization and may be propagated to become triticale. During the evolutionary process, polyploidy occurred in around 50-70% of angiosperms, including many agricultural plants (Chen *et al*., 2007) [10].

Many plant species, as well as many key crops, have polyploid genomes. The frequency of polyploid angiosperm species has been estimated to range from 30-35% to 80%. The bulk of estimates are within a 50% confidence interval (Soltis *et al*., 2000) [14]. Polyploidy levels in pteridophytes may be much greater, with some estimates in ferns surpassing 95% (Soltis *et al*., 2000) [14].

Several reviews on the origins and effects of polyploidy have been written throughout the last century (1950; Harlan and de Wet 1975; Levin 2002; Soltis and Soltis 1999, 2009; Ramsey and Schemske 1998; Otto and Whitton 2000; Yang *et al*., 2011; Ramsey and Ramsey 2014) [35, 13, 28, 22, 36, 21]. These studies have revealed a wealth of information regarding polyploidy, such as classification, frequency, and methods of genesis and ancient polyploidy events, as well as their ecological, genetic, and evolutionary relevance.

Polyploids frequently differ from their diploid parents and are thought to colonise better than diploids: While genome doubling has been observed in other major eukaryotic taxa, it is not like as prevalent as it is in plants. The discovery that many plant species, including several important crops, have polyploid genomes has aroused the interest of plant breeders in using artificial polyploidy as a crop improvement tool. Current research in this domain focuses on the applications of polyploidy in plant breeding, why it is more effective, techniques for polyploidy induction and detection, and some instances of economically relevant successfully induced polyploid crops. Polyploidy, or having more than two genomes per cell, is a common method of heredity. Based on chromosomal location, polyploidy is divided into two groups. Following are the limitations which affect the breeding programme in forage crops.

Limitations in breeding forages

1. Very small and delicate blossoms, such as berseem and guar, make hybridization problematic.
2. The majority of them are cross-pollinated, posing a maintenance issue.
3. Polyploidy in nature-chromosomal variations/abnormalities that result in varying levels of fertility, tough to manage/keep.
4. Many forage legumes have a limited germplasm base.
5. Grasses are typically apomictic in nature, which means they have distinct breeding processes.

6. long lived and perennial in nature and it require several years to assess their potential.
7. Insufficient seed output This makes it impossible to assess them for various economic characteristics in varied agro climatic zones.
8. Small seeds and fragile seedlings; establishing a good plant stand is challenging proper appraisal.
9. Most plants are grown in a combination, making evaluation difficult.
10. Evaluated under inadequate management conditions; given last priority.
11. Nutritional evaluation- time-consuming and expensive *in vivo* experiments are required and expertise is required.

Classification of polyploid

Euploidy

Euploids are polyploids with multiple copies of a species' full set of chromosomes. Based on the content of the ordering, euploids are frequently classified as autopolyploids or allopolyploids (Comai, 2005) [12].

Auto polyploids

Acquaah (2007) [1] and Chen (2010) [11] define autopolyploids as having more than two copies of the essential set (x) of identically arranged chromosomes. Crops that are naturally auto pollinated include lucerne, peanuts, potatoes and bananas. They occur spontaneously utilizing the body doubling approach (Acquaah, 2007) [1]. An example of an artificial autoploid developed through *in vitro* body doubling is seedless watermelon.

Allopolyploids

Allopolyploids are formed up of genomes from many species (Acquaah, 2007) [1]. They are the result of unedited gamete fusion across species or conjugation of two or more genomes followed by body doubling (Acquaah, 2007; Chen, 2010; Ramsey and Schemske, 1998) [1, 11, 28]. The most significant natural alloploid crops are strawberry, wheat, seed rape, and mustard (Acquaah, 2007; Chen, 2010) [1].

Impact of Polyploidy on Forage Yield and Contributing Traits

In compared to diploidy, induced polyploidy has multiple favorable impacts in a variety of forage species, including bigger leaves, greater herbage production, and gradual decay of heterosis (Table 2), increased plant height, persistence, quicker re-growth after grazing, and enhanced branching.

Aneuploidy

Polyploids that have one or more particular chromosome(s) added or deleted from the entire spectrum of chromosomes are called aneuploids. Many studies have showed that autotetraploid maize a unit aneuploids are responsible for 30-40% of the problem (Comai, 2005) [12].

Aneuploid classification where 2n diploid number of chromosomes and the number indicate excess or missing number of chromosomes.

Sr.no	Term	Chromosome number
1	Monosomy	2n-1
2	Nullisomic	2n-2
3	Trisomy	2n+1+1
4	Tetrasomy	2n+2
5	Pentasomy	2n+3

(Sources: Meru, 2012) [25].

Success of polyploidy

Considered vegetative copy and permanent habit to be essential aspects in polyploid establishment. During polyploid development, in combination with an outcrossing mechanism, to allow for sexual union (Across species, subspecies, races, populations, etc.). It was also useful for the convenience of new ecological niches. Another reason for polyploid success is the polyploid's bigger ecological amplitude in compared to diploids, stronger colonizing capacity, higher selfing rates, and magnified state, according to Soltis (2000) [14].

Increased Heterozygosity

According to Roose *et al*. (1976) [31], allotetraploid Tragopogon displayed mount state at isozyme loci, indicating a mix of distinct genomes. In the allotetraploids *Tragopogon mirus* and *Tragopogon miscellus*, respectively, thirty-third and forty-third of the loci examined were duplicated. When there is no clear citron divergence between the parental genomes, the body phase is duplicated; this data on duplicated genes is reviewed by (Wendel, 2000). Allopolyploids are basically heterozygous because of the non-segregating, mounted state.

Autotetraploids with tetrasomic genes exhibit higher levels of state than diploids, according to empirical studies. Soltis and colleagues (1989) [34] investigated multiple measures of genetic variety in wild populations of the two cytotypes (Soltis *et al*., 1989) [34]. Because of their manner of inheritance, polyploids are more likely to have higher levels of state than diploids.

Outcrossing rate of polyploids

Polyploid success has been attributed to greater colonizing capacity, which can include higher selfing rates than diploids. Sexual union depression is lessened in polyploids compared to diploids due to the buffering impact of extra genomes. The extra genomes hide dangerous alleles (Stebbins *et al*., 1950; Richard, 1986) [35, 29]. Allopolyploid and autopolyploid breeding depression is expected to be decreased (Barret *et al*., 1989) [6]. Two empirical investigations of sexual union depression in diploid and tetraploid fern couples were performed in an experiment. In the fern genus, 30-60% of selfed gametophytes of the diploid race generated sporophytes, whilst 100 of all selfed gametophytes of the diploid race produced sporophytes, although 100 of all selfed gametophytes of the diploid race produced sporophytes. while a hundred of the tetraploid race's selfed gametophytes produced sporophytes. Only four of the diploid race's selfed gametophytes generated conventional sporophytes in Lepisorus, but 98-100% of the tetraploid race's gametophytes produced sporophytes. This data was taken as evidence for lower sexual union depression in the tetraploid, indicating increased selfing rates. Masuyama and others (1990) [23].

Outcrossing rates have also been measured in Tragopogon diploid and allotetraploid species (Cook *et al*., 1999, 2000) [14]. Outcrossing rates were greater in the allotetraploid *Tragopogon mirus* (0.381 and 0.456 for two populations) than in the diploid parent *Tragopogon dubius* (0.068 and 0.242), but only in one of the two populations (Ownbey, 1950. There was no discrimination.

Allozyme variation can be used to predict outcrossing rates. This outline violates population genetic theory, and one explanation offered is that outcrossing rates were overestimated, particularly in *Tragopogon dubius*, due to few polymorphic loci in all populations.

Genome Rearrangements in Polyploids

Another important source of genetic innovation in polyploids is genome rearrangements. A genomic change in tetraploids relative to their diploid progenitors was investigated utilizing genome in situ hybridization (GISH). Tobacco (*Nicotiana tabacum*) is an allotetraploid hybrid of *Nicotiana sylvestris* and a diploid with the Tomentose T-genome. Using the GISH approach, several chromosomal rearrangements were found. As a result, most tobacco chromosomes are mosaics composed of areas from both parents. There is evidence that such genomic rearrangements may occur rather rapidly once the tetraploid in Brassica is established. Interspecific crosses between the parent plants *Brassica rapa* and *Brassica nigra*, as well as *Brassica rapa* and Brassica oleracea, produced offspring.

They compared the genomic structures of these crossovers' F5 descendants to their F2 grandparents and identified genetic divergence in just a few generations, with gaps as large as 10%.

Ancient Polyploidy and Gene Silencing

According to certain studies, ancient polyploidy is completely determined by chromosomal number (Stebbins *et al*., 1950) [35]. Polyploid plants, for example, have a basic chromosomal number of n =12 or higher. This criterion suggests that a significant number of angiosperm families and related rots angiosperm phylogeny are the product of ancient polyploid occurrences, some of which are now extinct. The Illiciales, for example, have n = 14, but the Lauraceae and Calycanthaceae of the Laurales have n = 12. The lowest chromosomal number in the Magnoliaceae is n = 19, and the family displays a range of values that are multiples of this base number. Some families with ancient polyploid beginnings, Table3 shows many families with ancient polyploid origins, as well as their chromosomal counts.

Because of the large chromosome counts of the basal angiosperm families, assuming base chromosome numbers for those groups of angiosperms is difficult. The chromosomal counts in all of the families in Table 3 are multiples of a single lower number. This happens because, following polyploidization, diversification happened at the new polyploid level, with subsequent episodes of polyploidy superimposed on the initial polyploidy level. This trend of diversified polyploid speciation calls into question the concept that polyploids are evolutionary dead ends.

Homosporous Pteridophytes

Homosporous pteridophytes are ferns such as Psilotum and Tmesipteris, as well as lycophytes and Equisetum, that generate the same type of spore rather than male and female spores. All of these species are derived from Devonian-era plant lineages. The typical gametic chromosome number for homosporous pteridophytes is n = 57; for angiosperms, it is n = 16. Despite possessing many chromosomes, homosporous pteridophytes exhibit diploid gene expression at isozyme loci (Soltis *et al*., 1988) [33]. There are at least two plausible causes for enormous chromosomal numbers and genetic diploidy. To begin, these are ancient polyploids that have experienced significant evolution. Recurrent Polyploid Formation's Genetic Implications: Second, they might have obtained a large number of chromosomes by another process, such as chromosomal fission.

Although morphological or cytologic variations across populations of some polyploid species give evidence of polyploid formation, most polyploid species were traditionally thought to be of different origin. The majority of polyploid plant species studied using molecular markers were found to be polyphyletic, having developed many times from a comparable diploid species. Polyphyletic polyploid species angiosperms and encompass each autopolyploid, for example, the genus *Heuchera grossulariifolia* (Soltis *et al*., 1989) [34]. The researchers used random amplified polymorphic deoxyribonucleic acid (RAPD) markers to test the hypothesis that isozymically identical populations of *Tragopogon mirus* with an equivalent plastid deoxyribonucleic acid haplotype and rDNA repeat were of separate origin, as were "identical" populations of *Tragopogon miscellus*.

Tragopogon mirus is divided into five populations, each of which possesses one isozyme Multilocus genotype (Soltis *et al*., 1999) [33]. Soltis *et al*. (1999) [33] discovered two isozyme genotype two populations. Every population has a RAPD profile (Two of which were polymorphic), hinting that each group may have a unique origin. Alternatively, *Tragopogon mirus* might be a combination of up to eleven lineages. *Tragopogon miscellus* demonstrated that each of the three was distinct and may have originated independently, increasing the number of genetically different populations.

Common Application of Polyploidy in plant breeding

Polyploidy is extremely common in both natural and man-made systems. There has been a great deal of experimenting with condition and its application during the course of agricultural science's hundred-year existence. Furthermore, certain elements are uniquely distinct and advantageous to human history. The environment benefits industrial items, crops, medicinal plants, beautiful plants, and so on. Some polypliod-valued plants are listed below.

Table 4: Examples of some polyploidy crops with their classification along with the commercial interest, origin (synthetic/natural), formation process (auto/all polyploidization)

Sr. no	Name	Scientific Name	Commercial interest	Formation process
1	Banana	*Musa acuminata*	Edible fruits	Autopolyploidy
2	Potato	*Solanum tuberosum*	Tubercle	Autopolyploidy
3	Sweetpotato	*Ipomea batatas*	Tubercle	Autopolyploidy
4	Tobacco	*Nicotiana tabacum*	Industrial	Allopolyploid
5	Kiwi	*Actinidia chinensis*	Edible fruits	Autopolyploidy
6	Rapeseed	*Brassica napus*	Oil seed	Allopolyploid
7	Plums	*Prunus domestica*	Edible fruits	Allopolyploid
8	Bread wheat	*Triticun aestivum*	Grain	Allopolyploid
9	Cotton	*Gossypium histurum*	Industrial	Allopolyploid
10	Coffee	*Coffea arabica*	Beverage	Allopolyploid
11	Sugarcane	*Saccharum officinarum*	Industrial	Allopolyploid

(*Sources*: Mariana, 2015).

Gigas effect of polyploidy

Ploidy affects the structural and anatomical features of plants. In general, the condition promotes increased leaf and flower size, stomatal density, cell size, and plastid count. These occurrences are referred to as the gigas result (Acquaah, 2007) [7]. It is involved in the production of vegetative crops as well as ornamental breeding, among other things. Physiological changes are also known to occur concurrently with order duplication. This is mostly due to metabolic alterations that increase secondary metabolites (Levin, 2002) [21]. This feature has found use in the breeding of medicinal herbs for the creation of prescription drugs, as a portion of the edible vegetative section, hybrid vigour resulting from interspecies crossings in allopolyploids is one of them.

Fig 2: Differences in leaf and bloom of a (Left) diploid and (Right) induced cowpea crop demonstrate the gigas effect.

Polyploids used for mutation breeding:

Polyploids can tolerate damaging cistron modifications following mutation, however they require a greater mutation frequency because to their massive genomes generated by their generous's duplicated state (Gaul, 1958) [16]. Polyploids' high mutation rates might be utilized to generate mutations in diploid crops that do not create enough genetic variation when subjected to agent treatment. This technique has been employed in the breeding of mutations in hot water plant species (Nut orchids). It is the first to produce autotetraploids using colchicine and the predominant use of X-rays. During this experiment, it was observed that autotetraploids had a mutation frequency that is 20-40 times higher than the equivalent diploid variety frequency with a large genome (Broertjes, 1976) [9].

Seedless fruit formation

Meiosis creates univalents and multivalents in autoploids, as opposed to diploids, which infrequently produce bivalents (Acquaah, 2007) [7]. During meiosis, autotetraploids, for example, can create bivalents, quadrivalents, and univalents. When univalents and trivalents reproduce, they create non-functional sterile gametes, which are most common in triploids and are hence sterile. That is why, rather than homologous chromosomes, sterile alloploids are formed during meiosis by the pairing of homeologous bodies from different genomes (Chen *et al*., 2007) [10]. As a result, inactive gametes were produced.

An allopolyploid requires diploid-like meiosis behaviour to determine viable gametes and complete fertility. Fertility issues in allopolyploids can also arise when crops with differing ploidy levels are crossed, resulting in multivalents. The seed lessness of triploids has proven advantageous, particularly in fruits. Watermelons and seedless bananas are examples of polyploid fruits. They want an absurdly high market price. Those are made intentionally by first making tetraploids and then crossing them with diploid fruits.

Polyploid fruits are crossed with an interesting diploid spore donor to line fruit. (Meru, 2012) [25]. An allopolyploid requires diploid-like meiosis behaviour to produce viable gametes and full fertility. Fertility problems in allopolyploids arise from multivalent formation when crops of different ploidy levels are crossed. The seedless nature of triploids has proven advantageous, notably in fruits. Triploid fruits include watermelons and seedless bananas. On the market, they are quite valued. Those are purposefully made by first producing tetraploids and then crossing them with diploid fruits. To establish fruit, the triploid fruits are mated with an appropriate diploid pollen donor. (Meru, 2012) [25].

Bridge crossing

Another breeding approach that takes use of polyploids' reproductive advantage is bridge crossing. When ploidy levels between two species cause sexual incompatibilities, intermediary crossings can be undertaken followed by chromosomal doubling to produce viable bridge hybrids. This method has been used to cultivate good tall fescue grass (Fescue arundinacea) from Italian ryegrass (2n=2x=14) and tall fescue (2n=6x=42) using meadow grass (Fescue pratensis) as a bridging species. By doubling the chromosomes in the superior child, the same procedure has been employed to repair heterozygozity in hybrids (Comai, 2005) [12].

Polypliody increase yield:

It boosted potato production in the cultivars Hagrai and Lalpakri at 70 DAPs, but late at 80 DAPs, varieties Shilbilati and Lalpakri yielded more and were treated with colchicine. Alam *et al*., 2011 [12]. The effect of polyploidy on the number of tubers at different DAPs was also examined, as was the effect of colchicine on the plant's fresh weight. The varieties Hagrai and Lalpakri produced more tubers at all intervals, which explained that higher fresh weight of plant generated more tubers owing to greatest assimilation and photosynthetic distribution, but the findings were statistically insignificant, as shown in the graph. In other types Alam, there is no relationship between fresh weight of plant and number of tubers per plant.

Conclusions

Plant assessments are one of the results of polyploids. Parental DNA sequencing, gene silencing, maternal and paternal impacts, increased ecological adaptability, and more heterozygosity all contribute to polyploidy success. Because to domestication, natural selection, and artificial section of polyploidies, polyploidy is found in a larger percentage of plants in nature and is a popular and highly productive plant.

Polyploid plants have more genomic and genetic diversity. Polyploidy allows plants to produce early variations, seedless fruits, sterile lines, prolific crops, resistance, and medicinal plants. Polyploidy is common in commercial crops including cotton, sugarcane, and sugar beets. Polyploidy therefore not only fights hunger but also eradicates poverty. Polyploidy is rather prevalent in forages. According to study, polyploid plants have better biomass output, persistence, regrowth after grazing, and resilience to abiotic stressors than diploid species. Genetically polyploid creatures contained many copies of alleles, which may help to increase allelic variation and give numerous evolutionary and adaptive advantages. Methods for induced polyploidy have been developed to increase biomass production and improve leaf quality.

Selection for balanced chromosomal pairing and disjunction may increase induced polyploidy stability. Induced polyploidy has also been utilized to increase the fertility of interspecific hybrids between incompatible species. The data showed that this polyploidy method might be utilized in fodder breeding with no negative consequences. Using this strategy, however, to produce unique cultivars. However, in order to use this technique for breeding novel cultivars, the selection cycles from early chromosomal doubling to stabilizing induced polyploidy and addressing both low fertility and loss of vigour in later polyploid generations must be minimized. A careful selection of anti-tubulin agent and its ideal dose may be necessary to enhance the frequency of viable polyploids with less damaging mutations. Background selection utilizing DNA markers can be used to choose genotypes with lesser mutagenic effects in a reliable manner.

While empirical approaches have proven problematic, confirmation of induced polyploid with balanced chromosomal pairing (bivalent or quadrivalent) may be necessary. Another approach for detecting the quantity of a duplicated genome is flow cytometry. However, pachytene analysis may be required to determine chromosomal pairing behavior. The stability of induced hybrids is being tested. Stability testing of induced hybrids under various agroclimatic conditions can be used to accurately establish the superiority of induced polyploids over equivalent diploids.

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