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Review Article

Factors Responsible for the Success of Anther Culture in Pepper Plant: A Review

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Abstract: Following the first successful plant regeneration from pepper in 1973, numerous research projects have been carried out to determine the factors influencing the induction of pepper plant regeneration and for the optimization of an anther culture in pepper. However, a variety of factors, are responsible for successful production of embryos. Critical factors for embryo production and plantlet regeneration are growing condition, donor plants growth conditions, selection of flower buds with microspores at the proper time and stage, and etc. This paper reviewed findings on three important factors responsible for the success of anther culture which is valuable for future research works and to understand factors in the development of haploid plants aimed at accelerating the breeding programs of pepper plants. One of the factors influencing the growth of anther culture is the effects of the growing season. The highest embryogenic yields were recorded with a genotype of growing in the summer season than that of winter season. The genotype of the donor plant is the second most crucial factor affecting androgenic potential, and it is essential to know the developmental time of pollen that can enhance anther culture efficiency. The breeding process for the development of plant varieties with high yield, disease resistance, and better quality attributes can be sped up by using a highly reproducible anther culture technique with optimized culture variables. In addition to the aforementioned variables, other factors would be taken into consideration for the effective development of another culture in pepper.

Keywords: Anther Culture, Donor Plant Age, Growth Conditions, Pepper

1. Introduction

Pepper (*Capsicum annuum* L.) is an important vegetable crop grown in temperate and tropical climates of the world. This is due to the fruits (high content of dry matter, vitamin C and B-complex content, minerals, essential oils, carotenoids, etc.), as well as their varied uses in the global food and beverage industries [14]. Haploid in pepper includes induction and regeneration of embryos from anthers or microspore culture. Plant regenerants with a single chromosomal set that develop from microspores of in vitro cultured anthers are suitable for genetic analysis investigation [2, 17, 22]. From gametes, haploid plants are created. Depending on how different species gamete cells react, androgenesis, gynogenesis, or parthenogenesis may be employed to produce haploid plants. Thus, the only chromosomes present in haploids are those seen in microspore or egg cells [7]. The chromosome set of a haploid plant is doubled, either spontaneously or artificially, resulting in homozygous doubled haploid (DH) plants. Doubled haploid technology involves both the production of haploid plants and the chromosome doubling process.

Today, the doubled haploidy (DH) technique is a common tool in biotechnology, genetic research, and plant breeding. With the aid of double haploid technology, the developmental pathway of a gamete cell can be altered such that it develops into sporophytic growth rather than mature pollen from microspore. Therefore, under specific stress conditions, a gamete cell can create a haploid embryo on its own [23, 24]. Due to the ineffectiveness of plant regeneration in the early investigations, research efforts in subsequent experiments focused on identifying the variables influencing the induction

of androgenesis [14]. After extensive experiments, it was shown that the success of androgenic anther culture depended on a variety of factors, including genotype, the growth conditions of the donor plant, pretreatment of the buds or anthers, the stage of microspore formation, incubation conditions, and others. This paper will review the experience, results, and knowledge obtained on three critical factors that are highly contributed to the success of anther culture on the base of internationally published research data. The information is tremendously helpful for future research works focused on experiments on anther culture and for understanding factors in the development of haploid plants aimed at accelerating the breeding programs of pepper plants.

2. Pepper Anther Culture

Anthers, in general, are very sensitive to in vitro culture. Guha and Maheshwari [11] reported the first successful anther culture in the 1970s using in vitro techniques. It has been applied to many species, primarily rice and tobacco. The first effective plant regeneration on pepper was reported by [10, 28] in India and in China, respectively. This was followed by identification of a number of factors influencing enhancement of pollen production and making the process more efficient and forming reproducible anther culture [6, 25]. Pepper anther culture provides a chance to select gametoclonal variations with novel genomic constitutions [12] and distinctive meiotic recombinants [9], as well as to find androgenic lines resistant to diseases and insect pest [3]. Haploid plants and DH lines are frequently used in investigations for gene mapping and the discovery of beneficial recessive mutations in pepper breeding programs [16].

The success of anther culture depend on various factors such as donor plant condition & maturity, genetic makeup of the plant, microspore developmental stages, panicle pretreatment, temperature and duration of pretreatment, culture media, environment and growth conditions.

2.1. Growing Conditions and Donor Plant Age

The effects of the growing seasons are one of the factors for anther culture development. The finding by [8] indicated that anthers from these two pepper genotypes gave different embryogenic responses to seasonal effects. The highest embryogenic yields were recorded with a genotype of growing in the summer season than that of the winter season. The study by Kristiansen and Andersen [15] the effects of growing conditions (donor plant temperature, photoperiod, and age on microspore embryogenesis) in pepper and revealed that temperature for donor plants and donor plant age affected the embryo formation from cultured anthers. The highest anther culture response was obtained with a condition of 268°C which is considered to be the ideal temperature for pepper.

According to the findings of the study by Ouyang *et al*. [21], even anthers from the similar genotype could respond differently to culture if the donor plants were grown in various environments. The physiological condition of the donor plants, which is influenced by factors including temperature, photoperiod, light intensity, and light, has an impact on flowering and pollen quality. The state of the microspores in culture determines how they respond even when the donor plant's genotype is excellent. Healthy donor plants cultivated in the field during an average vegetative period produce the best outcomes [27]. According to Ercan *et al*. [8] reported that the effect of donor plant age was a factor responsible for the success of anther culture of pepper. They got the highest number of embryos from plants of older ages (starting from four months old) in different cultivars, and concluded that anthers collected from older plants have given adequate embryogenic response when the ideal developmental stage is chosen. The results from Takahata *et al*. [26] revealed that microspores isolated from the buds of older plants produced more embryos than those from younger plants. The effect of inflorescence age showed a different pattern. Microspores isolated from fresh inflorescence buds were found to have a higher embryo response in older plants, whereas microspores isolated from older inflorescence buds had a larger embryo yield in younger plants.

2.2. Genotype of the Donor Plant

One of the most crucial elements affecting androgenic capacity is plant donor genotype, and there is significant variation in the androgenic response between genotypes subjected to the same inductive and cultural settings [4]. For many species, including pepper, the genotype of the donor plants is undoubtedly one of the key elements affecting the efficiency of androgenesis [15, 20].

Moreover, Niklas-Nowak *et al*. [18] concluded that the genotype of the donor plant is the essential factor determining the effectiveness of androgenesis in pepper anther cultures. They revealed that significant variations in each plant's androgenic response were seen across the genotypes that were evaluated. The three studied genotypes showed a distinct variation in the efficiency of androgenesis. The breeding lines of C. annuum of the F_2 hybrid plants showed the highest process efficiency, and in the hybrids 141 embryos were created, with the average androgenic efficacy accounting for 3.53% of them. On the contrary, plants from the line AT6 that was showed a decreased androgenic response, with embryos being generated between 0.5 and 3%. They concluded that the donor plant genotype plays a crucial role in determining the effectiveness of androgenesis in pepper anther cultures.

In addition to the difference in species, cultivars, or hybrid forms, individual plants of a single cultivar are also affected by the changes in genotypes for the androgenic response [15]. There were evident variation in the efficiency of androgenesis between pepper hybrid and individual plants of each genotype. The highest effectiveness of androgenic embryo development was observed for the cultivated form of C. annuum (ATZ1 9 PO) F_2. Most of the hybrid plants generated embryos at a rate greater than 5%, whereas the second C. annuum hybrid (ATZ1 9 CDT) F2's anthers produced approximately three times as few embryos and plants. A substantially reduced number of embryos were produced by anthers separated from interspecific hybrid flower buds [20]. This research on the

efficiency of androgenesis in pepper plants revealed significant variations in the individual responses of each hybrid studied, and clearly demonstrating that the genotype of the donor plant has a significant impact on the efficiency of androgenesis in pepper anther cultures. Similar research results by Hayati *et al*. [13] the genotypes were shown to differ significantly in terms of embryo induction and embryo conversion rates to complete plantlets. In the study, responsiveness to anther culture based on genotype ranged from 0.0% to 21.58%. The rate of conversion for embryo induction to plants varied between 0.0% and 100%. They concluded that appropriate protocols for each genotype should be determined experimentally. While developing androgenesis protocols to overcome genotype effects and recalcitrancy, it is important to develop genotype-independent techniques or to study genomic and gene editing technologies.

2.3. Developmental Stage of Bud and Anther

Pollen development stage can improve the effectiveness of anther culture. Understanding the stages of microspore development is still crucial since the morphological properties of buds and anthers and the ideal stages of microspore development for androgenesis may change for various plant species or even distinct cultivar species [1]. The best time for pepper embryogenesis to begin is during the flower bud stage, when the corolla and calyx are of the same length. According to Novak [19] for successful production of pepper haploid, use anthers with uninucleate microspores, obtained from buds measuring between 2.6 and 5.0 mm.

The results of Mangal and Srivasatava [16] demonstrated that the size of the flower bud in the range of 6-7 mm was the ideal developmental stage for initiating androgenesis in pepper. Similarly, anthers are around 450 m long, the corolla is somewhat longer than the calyx or covers 90% of the corolla, and the green anthers have just faint purple pigmentation at the apical end were the ideal developmental stage for initiating androgenesis in pepper. Further, it was observed that when buds and anthers were selected based on these parameters they contained almost 90% microspores in the vacuolated stage. The bud size and morphological characteristics of the buds and anthers were defined and the microspore stages were determined. The buds 5 mm in diameter and 7 mm in length were determined to contain microspores at the uninucleate and 1st pollen mitosis stages. At this stage, the length of the corolla was about the same as or slightly greater than that of the calyx [5]. The late uninucleate and early binucleate stages are ideal for the creation of a successful experimental anther culture system. It is recommended that in vitro androgenesis always should begin with thorough cytological examination so that flower bud size and morphology correspond to the proper developmental stages for anther production [14].

3. Conclusion

Since, the first successful plant regeneration from pepper in 1973, numerous research projects have been carried out to determine the factors influencing the induction of pepper plant regeneration and for the optimization of an anther culture in pepper. Pepper anther culture is a desirable method for growing haploid plantlets. However, a variety of factors, including the donor plant's maturity, genotype, the stages of microspore development, the pretreatment of panicles, including their temperature and length, culture media, and growth circumstances, all play a role in whether or not a culture is successfully produced. This implies that not all anther embryos can mature into fully grown plants, and not all acclimatized plants are viable. Critical factors for embryo production and plantlet regeneration are growing condition, donor plants growth conditions, selection of flower buds with microspores at the proper time.

One of the factors influencing the growth of anther culture is the impact of the growing seasons.

The highest embryogenic yields were recorded with a genotype of growing in the summer season than that of winter season. For a genotype that grew better in the summer than the winter, the largest embryogenic output was seen.

The donor genotype of the plant is the second most crucial factor affecting androgenic potential, and genotypes subjected to the identical inductive and cultural conditions exhibit markedly different androgenic responses. The capacity of pepper genotypes that gave response with embryogenesis has a range of variation from good to poor and nonresponsive genotypes. Understanding the stages of microspore formation is crucial since different plant species or even cultivars may differ in the physical traits of their buds, anthers, and the appropriate stages of microspore development for androgenesis. The breeding process for the development of plant varieties with high yield, disease resistance, and better quality attributes can be sped up by using a highly reproducible anther culture technique with optimized culture variables. In addition to the aforementioned variables, other factors would be taken into consideration for the effective development of anther culture in pepper.

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