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Review Article

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EFFECTS OF COLCHICINE AND ITS APPLICATION IN COWPEA IMPROVEMENTS: REVIEW PAPER

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Abstract

Availability of genetic diversity and genetic variation is the heart of any breeding program which plays a critical role in developing, well-adapted and improved varieties. Where genetic diversity is insufficient in cowpea, new material needs to be accessed or new variation can be created through induced mutation. Chemical mutagenesis has been a beneficial technique in the improvement of yield characters in crop breeding which has low genetic variability. Mutation breeding has been successful in many crop species but least applied in legumes like cowpea. It has also been exploited to enhance genetic variability in cowpea (*Vigna unquiculata* L. Walp.); as it is an important legume in the tropical and subtropical regions of the world. Mutation breeding is regarded to be a shortcut breeding technique, which has produced new and high yielding varieties through heritable changes in genetic constitution of characters in some leguminous crops. Colchicines treatment produced shortened internodes, deformed leaves, and chlorophyll mutants in different crop plants. It has the capacity of inducing polyploidy; especially tetraploid on seeds of cowpea, which obviously could be harnessed for further breeding and improvement of the cowpea. This paper aims to review the effects of colchicines on agronomic parameters of cowpea.

Key Words: Mutation, Mutagenesis, Colchicines, Cowpea, Agronomic traits.

INTRODUCTION

Cowpea (*Vigna unguiculata* L. Walp) is a diploid species with 2n=2x=22 chromosomes. It is one of the most important food and forage legumes in tropic and sub-tropics areas of the world (Singh, 2012). Cowpea is mostly cultivated by small scale farmers, usually intercropped with various cereal crops such as leaf vegetables, maize, millet, sorghum, beans, pigeon peas, bananas and others. The production of cowpea around the world is primarily as seed, as a vegetable (for leafy greens, green pods, fresh shelled green peas, and shelled dried peas) and as cover crop. This legume can also a major source of protein (22-24 %), carbohydrate (50-67 %), starch, vitamins and minerals as unripe pods or dry seeds (Pavadai *et al*., 2009) and as fodder (Baudoin, 2001; Tarawali *et al.,* 2002). Cowpea has great ability to fix atmospheric nitrogen through its root nodules, and grows well in poor soils with more than 85% sand with less than 0.2% organic matter and low levels of phosphorus (Singh, 2003). Since, cowpea is shade tolerant and compatible as an intercrop with cereal crops helps to prevent build up of diseases incidence, insect pests and weeds. Cowpea is therefore regarded as a truly a multifunctional crop, providing food for man, feed for livestock and serving as a valuable and dependable revenue generating commodity for farmers and grain traders (Timko and Singh, 2008).

However, the general grain yield of cowpea especially in marginal areas is still low (Ajayi and Adesoye, 2013) and no single variety can be suitable for all growing conditions; whereby varietal requirements in terms of plant type, seed type, maturity and use pattern are highly diverse from region to region and these makes cowpea breeding programs more complex than any other crops. There are several options for breaking the yield barrier in crops. Induced mutation is one of the options to increase the genetic variability, produce many cultivars with improved economic value, used to study of genetics character and plant developmental phenomena in most crop plant (Horn and Shimelis, 2013).

It has been used to generate genetic variability and has been successfully utilized to improve yield and yield components of various crops in the world (Khan and Wani, 2006). This genetic variability is required for crop improvement as variability that can be exists in all organisms including all crop plants has been generated by mutation and subsequent recombination (Aliero, 2006; Bolbhat *et al*., 2012). Mutagenesis in plants can be achieved by using physical agents (ionizing and non-ionizing radiations) or chemical mutagens. Nevertheless, several practical problems with chemical mutagens have been identified, which includes soaking of seeds, penetration of the relevant target cells, safety of handling and disposal among many others (Pande *et al*., 2012).

Chemical mutagenesis is a coherent tool used in mutation breeding program for creating new alleles and is relatively cheap to perform and equally usable on a small and large scale (Laskar and Khan, 2014a). Among chemical mutagens, colchicines treatment is one of the best tools of inducing and enhancing genetic variability in crops within a very short time span (Gnanamurthy *et al*., 2013). This chemical is known to inhibit mitosis in a wide variety of plant and animal cells by interfering with the orientation and structure of the mitotic fibers and spindle fiber (Khan and Goyal, 2009). Since chromosome segregation is driven by microtubules, colchicine is therefore applied to interfere with mitosis to induce polyploidy and mutations in plant cells. While polyploidy is fatal in animal cells, it is usually well tolerated in plant cells and mostly results in fruits and seeds that are larger, hardier and faster growing and more desirable (Ranney, 2006). For the above mentioned reasons, this type of genetic manipulation is frequently used in breeding plants to create

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genetic variability. Several high yielding variants of crop plants have been developed using colchicines. The ability of this chemical to induce polyploids in crop species depend on the chemical concentration, duration of exposure and species of crop plant being investigated (Udensi *et al*., 2012a-c). Polyploidy population display heterosis relative to their parental species and may also display novel variation or morphologies that may contribute to the processes of speciation and ecoloniche exploitation (Comai, 2006).

So that, the main advantage of mutational breeding is the possibility of improving one or two quantitative characters without changing the rest of the genotypes. And also, has a great potential and serve as a complementary approach in genetic improvement of crops (Mahandjiev *et al*., 2001). Therefore, mutation breeding has been playing a key role in self-pollinated crop with limited variability. In this context, it is to be noted that many workers have observed in cowpea (Dhanavel *et al.,* 2008), in black gram (Thilagavathi and Mullainathan, 2009) and soybean (Pavadai *et al.,* 2010) developed plants by mutation on breeding. Furthermore, induced mutation is recognized as a valuable supplement to conventional breeding in crop species improvement programs, but has been least applied in grain legumes like cowpea (Dhanavel *et al*., 2012) till know. So, the objective of this review paper is to realize the effects of colchicines and its application in cowpea improvements

Mutation

Mutations are changes in the DNA base sequence of a cell's genome caused by radiation, viruses, transposons and mutagenic chemicals, as well as errors that occur during DNA replication. It can be deletion of bases, or molecular changes within the physical limits of the gene. Mutation may involve the rearrangements, loss, or duplication of chromosome segments (Bertam, 2000). It can be detected because of some phenotypic (agronomic) changes in plants. A visible change in a morphological characteristic such as, pericarp colour, leaf marking, chlorophyll deficiency, endosperm texture, spike density, etc, is most easily identified. Mutation changes in quantitative plant characteristics, such as, physiological activity and chemical content. Their effects may require exacting measurements, often on a population of plants rather than a single plant (Laskar and Khan, 2014a).

Induced Mutation

Induced mutations have played a very vital role in altering the genetic make-up of genotypes not only at a chromosomal but even at a molecular level (Alan, 2007). Induced mutation both in seeds and vegetative propagated crops is one of the techniques employed in the improvement of traits of economic plants part. Moreover, polyploids exhibits more heterozygousity than their diploid counterparts, the degree of this condition could possibility be the underlying factor in their growth performance and adaptability to environmental stressors (Ranney, 2006). It facilitates the isolation, identification and cloning of genes which would ultimately help in designing crops with improved yield, increased stressed tolerance and longer life span as well as reduced agronomic inputs usage (Ahloowalia and Maluszynski, 2001).

Spontaneous Induced Mutation

A spontaneous mutation is one that occurs in nature, while an induced mutation results from the action of a mutagenic agent. It is the mechanism by which new genetic traits arise in nature. Spontaneous mutation may result from the activity of mobile genetic elements (transposons) that can move around to different positions within the genome of a single cell and affect the activity of gene in which they are inserted (Wessler ,2006). Most spontaneous mutations occur in very low frequencies of individual gene. Since spontaneous mutation rates are very low. The selection for economically useful spontaneous mutants still takes place with some levels of success (Ahloowalia *et al*., 2004)

Spontaneous mutations on the molecular level can be caused by:

Tautomerism: A base is changed by the repositioning of a hydrogen atom, altering the hydrogen bonding pattern of that base resulting in incorrect base pairing during replication.

Depurination: Loss of a purine base (A or G) to form an apurinic site (AP site).

Deamination: Hydrolysis changes a normal base to an atypical base containing a keto group in place of the original amine group. Examples include C → U and A → HX (hypoxanthine), which can be corrected by DNA repair mechanisms; and 5MeC (5-methylcytosine) → T, which is less likely to be detected as a mutation because thymine is a normal DNA base.

Slipped strand mispairing: Denaturation of the new strand from the template during replication, followed by renaturation in a different spot ("slipping"). This can lead to insertions or deletions. A covalent adduct between benzo[a]pyrene, the major mutagen in tobacco smoke, and DNA

Artificially Induced Mutation

Artificially induced mutations have been used more frequently to raise the frequency of mutations and variations, which can be amplified through chemical mutagens as for instance alkylating agents (ethylmethanesulfonate and methyl-methanesulfonate), colchicines, sodium azide, as well as physical mutagens, such as ionizing radiations (Predieri, 2001). Chemical mutagens are more effective than physical ones. They enhance genetic variability in higher plants for successful breeding programs in vegetatively and sexually propagated plants (Dhanayanth and Reddy, 2000; Bhat *et al*., 2005)

Though, chemical mutagenesis has been proved as fundamental in the improvement of crop plants. It is a simple technique used to create mutation in plants for their improvement of their potential agronomic traits, particularly for traits with a very low level of genetic variation (Szarejko and Forster, 2007). Chemical mutagens is one of the most important tools also used to study the nature and function of genes, which are the building blocks and basis of plant growth and development, thereby producing raw materials for genetic improvement of economic crops. Chemical mutagens generally produce induced mutations which lead to base pair substitutions, especially GC→AT resulting in amino acid changes, which change the function of proteins but do not abolish their functions as like deletions or frame shift mutations. These chemo mutagens induce a broad variation in morphological characters when compared to normal plants (Adamu and Aliyu, 2007).

Mutagenic Effectiveness And Efficiency Colchicine

The presence of genetic variability is necessary for the sustainable crop improvement. The variability available to the breeders comes from spontaneous or artificially induced mutations. The artificial induction of mutation in a crop species is achieved through the use of physical and/or chemical mutagens that enlarge the mutation frequency, when compared to the spontaneous occurrence. Almost all mutagens have the property of reacting with DNA and thereby bringing about changes in nucleotide sequences. However, the mode of action of each mutagen is distinct. Besides, a mutagen may effectively bring about mutations, but the accompanying undesirable effects like lethality or sterility may decrease its efficiency. Thus, in order to exploit induced mutagenesis for crop improvement, the basic studies on effectiveness and efficiency of a mutagen in a crop are necessary to recover high frequency of desirable mutations (Badere and Chaudhary, 2007). Mutagenic effectiveness is an index of the response of a genotype to the increasing doses of the mutagen, whereas mutagenic efficiency indicates the extent of genetic damage recorded in the M2 generation in relation to the biological damage caused in M1 (Wani, 2009).

Dose Effect Colchicines on Legumes

Colchicine affects the microtubule depolymerization through the formation of colchicines complex. The intensity of this complex should be proportional to the polyploids inducing capacity of this mutagen. Besides, the dose required for high mutation efficiency of a chemical mutagen is depends on the properties of the agent, the solvent medium and the biological system. In general, the dose of a chemical mutagenic treatment comprises several parameters, of which the most important are concentration, duration of treatment and temperature during treatment (Udensi and Ontui, 2013). Artificial induction of mutation by colchicines leads to the alteration of plant genome integrated by environmental signals as reported by Uno *et al*., (2001) probably by increasing the rates of cellular division and expansion at their meristematic regions. This on the other hand can be viewed as the mechanism through which colchicines-induced in all mutants emerged. The mutagen might have probably influenced the activities of cytokinin which is paramount importance in the fundamental processes of plant development including cell division and morphogenesis. The damage to the biological materials might be considered as an indication of the mutagenic effects and the level of dose. In colchicines treated plants, along with diploid cells, polyploid cells also have been observed, mostly tetraploid cells and rarely triploid (3n), haploid (n) and pentaploid cells (5n) in the root tips of legumes and cereal crops. The details about the percentage occurrence of mitotic aberrations in the root tip cells particularly broken metaphases, anaphasic laggards and anaphasic bridges in various control colchicine treated plants (0.12, 0.14 and 0.16 %) and period of expose. It has been clearly shown in a number of plant species that the effect induced, varies with the varying mutagens and with the variation in mutagen doses. Thus selecting a mutagen and its optimum dose for a genotype in any plant species is an important step in mutation breeding program.

Induced Mutation Breeding In Grain Legume Crop Improvement

Induced mutation breeding which has been recognized as a valuable supplement to conventional breeding in crop improvement has been least applied in grain legumes (Pande *et al*., 2012). But, legumes are found to be well suited for genetic improvement through mutation breeding due to their evolutionary selection history. It has been shown that most legumes have lost many alleles for high productivity, seed quality, pest and disease resistance in the process of adaptation to environmental stress. Valuable progress has, however, been reported on the improvement of nutritional quality of some legumes by induced mutation (Ahloowalia *et al*., 2004). Mutation breeding offers the possibilities of recovering some of the lost alleles and useful variability in cowpea, especially improved yield and disease/pest resistance. Mutation techniques have contributed significantly to plant improvement worldwide, and have made an outstanding positive impact on productivity and economic value of some legume crops.

Morphological Characterization Colchicines In Agronomic Traits of Cowpea

Variability of quantitative traits influencing yield have been greater in mutagenic progenies than in control of cowpea. Ability of mutagens to enter the cell of the organisms to interact with DNA produces the general toxic effects associated with their mutagenic properties (Mensah *et al*., 2006). It has been widely proved that chemical mutagens induce physiological damages (injury), gene mutations and chromosomal aberration in M1 individuals which can be detected and measured from seed germination or emergence of seedlings, survival reduction (lethality), plant height reduction (injury) and fertility reduction or sterility (reduction in pod and seed formation) (Kumar *et al*., 2009) which might not be restricted to M1 generation. Growth inhibition after mutagenic treatment has been a known phenomenon in most legume crop plants. Reduced growth rate after colchicines treatments has been linked to signs of polyploidy and successful treatments of Colchicines. The increased in branching of the treated plants have many number of nodes, which determine the growth habit of cowpea (Rauf, 2006).

Daye to seedling emergence was increased significantly as the concentration of the mutagen increased. This was confirmed by Mensah *et al* (2005) in cowpea, Udensi *et al* (2012a-c) in cowpea and cajanus pea the gama seed irradiation and prophos methyl treatments, respectively. The delay in seedling emergence could be attributing chiefly to physiological disturbance which might have probably affected some biological pathway in the treated seeds. This might have delayed seedling emergency and finally reduce the germination percentage. It is probable that the seeds soaked in higher concentration of colchicines have to readjust in response to the treatment thus delaying the time it took the seed to emerge (Brisibe *et al*., 2011)

CONCLUSION

The morphological mutant characters can be utilized for identification and characterization of cowpea genotypes. Colchicines induced marked vegetative growth leading to the formation of large plants with more number of leaves, branches per plant and seeds. Colchicines caused noticeable differences in quantitative traits like twining tendency, internodes length, plant pigmentation, testa texture and leaf color in cowpea.

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