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CHROMOSOME CLUMPING IN VIGNA UNGUICULUTA (L) WALP ROOTS TREATED WITH PHOSTOXIN FUMIGANT

HAUWA'U ISA[1], ANDREW OSVMETE[2] & KELECHUKWU CHRIS EGBUCHA[3]

[1,2]Department of Plant Science and Biotechnology, Michael Okpara University of Agriculture, Umudike, Nigeria

[3]Department of Plant Science, Nassarawa State University Keffi, Nassarawa, Nigeria

ABSTRACT

The effects of treating seeds and germinating root tips of Vigna unguiculata (L) Walp with Phostoxin fumigant, on its mitotic chromosomes were investigated. Healthy grains of Vigna unguiculata were presoaked overnight in a solution made by dissolving one pellet of the fumigant (4gm) in 100mls of tap water. The same solution was used to moisten cotton wool on which the seeds were germinated in a petri dish. A control experiment was set up using ordinary tap water. Germinating root tips were pretreated in Colchicine solution and fixed in acetic ethanol. Result showed the chemically treated root tips exhibited clumping of the metaphase chromosomes. Comparison with the untreated root chromosomes indicated that the effect of the chemicals present in the fumigant used, reasonable impaired the process of mitosis. This implied that growth and development of germinating seedlings treated with Phostoxin could be hampered.

KEYWORDS: Phostoxin, Vigna, Mitosis, Clumping, Colchicine, Phosphine.

INTRODUCTION

Cowpea is the most economically important indigenous African Legume crop (Langyintuo, et al., 2003). In West and Central Africa, the crop cowpea plays a very vital role in the livelihood of several millions of people, serving as food for Humans, animals and for cash. It is appreciated by both Urban and Rural dwellers and many traditional African meals and seasonings are prepared from cowpea, including some child weaning foods (Lambot, 2002). It is regarded as a hungry season food due to its early maturing time (usually before other crops like yam, cassava, sorghum etc. (Okike 2000). The grain serves as an affordable source of protein being made up 20-30% protein (Adetula, 2006).

Post harvest loss of cowpea is the most common constraint to the production of cowpea. The bruchid weevils of cowpea are the main culprit. It not only attacks the seeds under storage condition but can fly to the field to infect the pod. They are cosmopolitan pests that cause considerable economic damage (Profit, 1997). Approximately one-third of the food produced (about 1.3 billion ton), worth about US $1 trillion, is lost globally during postharvest operations every year (Gustavsson et al., 2011). According to the World Bank report, sub-Saharan Africa (SSA) alone loses food grains worth about USD 4 billion every year (Zorya et al., 2011). For cowpea as a crop, Murdock (2002) observed that even when the crop has been harvested, the grain has still not escaped its insect enemies.

Due to the enormity of damage done by the bruchid weevils on post harvest cowpea grains, many chemicals are available as storage chemicals. Some of these chemicals are recommended with caution, an aspect which the average African rural farmer does not observed due to an array of factors, ranging from sheer ignorance to inability to comprehend the contents of the instruction manual. Phostoxin, a highly toxic inorganic fumigant, is one of such chemicals. It is a trigonal pyramidal molecule with C3v molecular symmetry (Ntoukam et al., 2000).

Cytological aberrations in plants serve as an excellent monitoring system for the detection of environmental chemicals that may pose a genetic hazard (Nilan and Vig, 1976). The aim of the present research is to determine the effect of phostoxin treatment on mitotic chromosomes of cowpea (Vigna unguiculata (L.) Walp).

MATERIALS AND METHODS

The experiment was conducted at the postgraduate Laboratory of Department of plant science and Biotechnology, College of Natural Sciences Michael Opara University of Agriculture, Umudike, Abia State, Nigeria. Some pellets of phostoxin fumigant were obtained from a commercial Agricultural chemical store in Umuahia town, Abia State. One pellet (containing 55% aluminum phosphide mixed with ammonium carbamate) was dissolved in 100 ml of tap water. In the control, no chemical was added to the water.

Some grains of Vigna unguiculata were soaked with the solution of phostoxin, for 24 hours. For the control, the seeds were soaked in tap water for 24 hours. The solution used to soak each group of seeds overnight was also used to moisten cotton wool for germinating the particular bean seed. After 3-4 days the germinating root tips were excised and pretreated with 0.05% colchicines solution for 5 hours. They were fixed in Acetic alcohol 1:3 v/v for 24 hours and stored in 70% ethanol till cytological investigation. To prepare the root tips for hydrolysis they were soaked in distilled water for 15 minutes and hydrolyzed in INHCL. At 60^0C.After cooling to room temperature, the hydrolyzed roots were stained and squashed in acetic orcein and viewed under low and high magnifications and good spreads were photomicrography.

RESULTS

The chromosome complement of the root tips soaked and germinated in ordinary water (control) appeared regular at metaphase with distinct recognizable 22 chromosomes made up of 11 pairs. The morphology of most of the homologues was clear with distinct centromeres. Figure 1 shows the full complement of the karyo type of the untreated root tips.

For chromosomes of the treated root tips, the metaphase stage of mitosis showed clumped chromosomes whose individual outline could barely be visible (Figure 2). The clumping configuration was done in different cluster groups which appeared as multiple associations (Nair and Ravindran, 1994).

Figure 1: Full Mitotic Complete of the Untreated Root Tip

Figure 2: Mitotic Plate of the Treated Root Tip Showing Clump Clusters

DISCUSSIONS

The untreated root tips of Vigna unguiculata (L.) Walp Displayed normal karyotype with regular diploid chromosome status of 2n=22. This result is in agreement with other reports on the species (Alam et al., 2007; Adetula, 2006; Galasso et al., 1996; Venora and Padulosi, 1997). Adetula (2006) reported some 20% of cells in V.unguiculata Ssp. Dekindtiana Var. pubesces (TV.NU 110-3A) showing diploid chromosome number of 23 in the karyotype. The four major morphological categories of chromosomes were represented. Thus, metacentrics, submetacentrics subtelocentric and Telocentrics were present in the following proportions: seven metacentrics, one submetacentrics, one subtelocentric and two telocentrics. The implication of this is that the karyotype of the untreated root tip of Vigna unguiculata is majorly symmetrical.

The cells of the root tips treated with phostoxin fumigant showed a level of clumping which would not permit clear display of the morphological outlines of the individual chromosomes (Plate 2). For the same reason chromosome count, as well as identification and characterization of the metaphase chromosomes was impossible. This abnormality, no doubt was induced by the action of the chemicals used in treatment i.e. aluminum phosphide and ammonium carbamate which are the active components of phostoxin fumigant. Large arrays of chemicals have been found to have effects on chromosomes. There is no uniformity in either the structure of these chemicals or the nature of their actions (Swanson, 1956). The mitotic poisons like colchicine induce mitotic blockage or destruction of the spindle. Others induce chromosome stickiness while some induce breakage. In plants the basic condition for most of these chemicals is penetration and this is usually through the root tip (Levan 1938).

In the present investigation, chromosome clumping seems to be the only visible cytological effect of these chemicals on the cells. The mode of action of the chemicals on mitotic cells seems to be that mitosis is allowed to initiate but arrested midway, probably at metaphase or early anaphase stages. This may mean that the aluminum phosphide, the ammonium carbamate or the phosphine gas, generated by the action of water on the former, interferes with one or more of the Cyclin dependent kinases (CDKs), which regulate the pace and progression of mitosis in higher plants. In attempting to provider an explanation on the mode of action of chemical mutagens, Shukla and Chandel (2007) observed that mutagenic chemicals react directly with the genes. Consequent upon this reaction, the gene 'makes a mistake' when it replicates.

This situation more often than not, leads to an error in physiological activity. Reddy and Jappa (2003), while describing chromosome clumping and stickiness in meiotic cells of Mulberry genotypes observed that these phenomena may have arisen from partial dissociation of the nuclear proteins and alteration of their pattern of organization or due to depolymerization of nucleic acids. Darlington, (1942) had earlier attributed chromosome clumping to disturbed action of nucleic acid metabolism of the chromosomes. With the degree of clumping observed in the treated root tips (Plate 2), normal completion of mitotic cycle would suffer serious impairment.

CONCLUSIONS

The level of chromosome clumping induced as a result of treatment of the root tips of this crop calls for more attention to be directed in the area of the extent of cytological damage that storage chemicals such as phostoxin may be capable of inflicting on grains which they are used on. Also, since mitosis is mainly concerned with growth in multicellular organisms, the effect of these storage chemicals on germination and breaking of dormancy of the affected grains ought to be further investigated.

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