

Biotechnology Congress 2015 : Mutagenic effects of sodium azide and gamma rays on callus development and silymarin production in *Silybum marianum* (L) Gaernt - Raoufa A.I. Abdel Rahman - City of Scientific Research and Technology Applications

Raoufa A.I. Abdel Rahman¹, Elfaramawy A M², Badr A³ and Amer M A¹

¹City of Scientific Research and Technology Applications, Egypt

²Tanta University, Egypt

Plants are important source for natural drugs and modern medicines; it is estimated that approximately one quarter of prescribed drugs contain plant extracts or active ingredients obtained from plant substance. *Silybum marianum* (L) Gaernt is a member of the family Asteraceae native to the Mediterranean area. The plant extract has anticancer, anti-inflammatory, antioxidant, hepatoprotective, antidiabetic and immune-modulatory effects. In the present study, callus cultures obtained from leaf explants using MS medium supplemented with 1 mg/l BA and 1 mg/l 2,4-D. Effects of sodium azide and gamma rays on callus growth and silymarin production were tested. Callus treated with 1,2,3,4 or 5 mM NaN₃ for 1 hour or exposed to γ -radiation at doses of 10, 20, 30, 40 or 50 Gray. Results indicated that the fresh weight of treated callus decreased by increasing the dose of NaN₃ and γ -rays. The chemical composition of silymarin was determined by HPLC. Sodium azide and gamma rays could induce the production of isosilybin A that was not detected in control callus. They reduced or enhanced the production of other silymarin components. Differential display technique was used to identify differentially expressed genes associated with silymarin production. Nine unique cDNAs over-expressed in treated callus and representative of suspected genes that expressed due to mutagen stress were purified and sequenced. Most of the obtained nucleotide sequence and corresponding amino acid displayed 100% similarities to chalcone synthase that is a key enzyme in flavonolignans biosynthesis. The overexpression of chalcone synthase may be the cause of enhanced production of some silymarin components. Transformation is an unexpected heritable change in a living being. Which might be basic or useful, however for the most part basic changes happen. It is created by changes in base grouping of quality and it very well may be suddenly or misleadingly both in

seed and vegetative spread yields. In any case, seed is the most regularly utilized material for mutational investigations since it can endure states of being. Actuated transformation has been built up as a significant apparatus for development of specific attributes in the current germplasm. Changes are of two sort viz., normal and counterfeit or initiated transformation. Recurrence of regular transformation is low and thus, counterfeit change with utilization of mutagens is followed to show signs of improvement hereditary fluctuation. Different kinds' of synthetic compounds fit for initiating change in plants had been discovered. They are Ethyl Methane Sulphonate (EMS), Methyl Methane Sulphonate (MMS), diethylsulphate (DES), Ethylene Imine (EI), Hydroxyl Amine(HA), n-nitroso-n-ethyl urea (NEU), Nitrous Acid(NA), sulfur mustard, 5-bromouracil, Sodium Azide (SA) and so forth [5]. Impressive advancement has been made on incite changes of okro [*Abelmoskhusesulentus* (L.)Moench] for development of their specific characters, yet point by point contemplates are deficient. Attributes like germination, dust sterility, plant length, natural product length, endurance to development and mortality are greatly influenced by mutagenic treatment. In this manner, an endeavor has been made to contemplate the impact of mutagenic treatment on agronomic characters of okro. Initiated transformations have as of late become the subject of biotechnology and atomic examination prompting depiction of the structure and capacity of related qualities. Actuated transformation in plant is a successful apparatus for crop improvement [1].The mutagenic impacts of sodium azide have been archived in past reports. [6] Reported that sodium azide is an intense mutagen in grain and prompted chlorophyll insufficiency just as a wide scope of morphological and physiological freaks and [3] detailed that gamma beam mutagenesis can be relied

upon to yield serious phenotypic high extent of valuable transformations with ordinary yielding properties. In plant cells; the core is viewed as the chief site of harm by ionizing radiation [1]. Materials and Methods Plant Source and Mutagen Treatment Two improved assortments (Table) of Okro, *Abemuscusesculatus* L. Moench seeds were gathered from Institute of Agricultural Research (Plant science office) and a neighborhood assortment was bought in Sabo principle showcase Zaria and taken to Center for Energy Research and Development, ObafemiAwolowo University Ile-ife ($7^{\circ} 28'N$ and $4^{\circ} 32'E$) for radiation with Gamma cell 220 Cobalt 60 (Co60) some portion of the seeds were treated with Sodium azide in the lab in Biological Sciences division, Ahmadu Bello University (A.B.U.), Zaria. ($11^{\circ} 04'N$ and $7^{\circ} 42'E$) The Sodium azide utilized for this examination was gathered from Biochemistry office A.B.U. zaria. Planting of the Seeds The trial was sorted into two treatment gatherings. For example sodium azide and gamma beams. In first class, seeds were presoaked in cushion answer for two hours at that point absorbed sodium azide arrangements of 1.0mM, 2.0mM, 3.0mM and 4.0mM for one hour after which the rewarded seeds were washed in faucet water to expel abundance synthetic compounds and exudates from the seeds and in second classification; 20,40,60,80 and 100 kR dosages of gamma beams utilizing Co60 as a source in gamma chamber. The seeds were watched every day until greatest germination was accomplished. Planting of sets of seeds rewarded Sadium Azide, those rewarded with Gamma beam and the control seeds were done in Botanical nursery, Ahmadu Bello University Zaria, utilizing the open nursery to examine the impact of the two mutagens on each arrangement of okro seeds. Each plot comprised of five columns and each line was four meters in length with the intra line dispersing of 50cm and entomb line separating of 75cm separated in three replications. Eight slopes of two seeds each were planted per column. The format follows 3×5 Randomized Complete Block Design (RCBD).

Information Collection The development of plant was assessed according to the hour of blossoming, development and changeability in plant advancement inside and between medicines. The accompanying information were gathered: rate germination,

seedling tallness, and leaf length, endurance at development, organic product length and stature at development.

Factual Analysis The information gathered were exposed to the accompanying factual techniques for examining the information: Mean (normal) rates, investigation of fluctuation (ANOVA) and t-test was utilized to decide whether there was any huge distinction between the two mutagens.

Results and Discussion The T-Test Between The Two Mutagens Shows There Is Significant Difference In The Leave Length And Fruit Length For Nhae47-4. There Was No Significant Difference Between The Effects Of The Two Mutagens For The Other Parameters Of The Nhae47-4. Additionally, There Was No Significant Difference Between The Effects Of The Two Mutagens For The All Parameters Of Beru And Jokoso (Table 1). This Shows That The Two Mutagens Have Similar Effect On The Okro Varieties.

Biography

Raoufa A I Abdel Rahman has completed her PhD in 2003 at Alexandria University, Faculty of Agriculture, Genetics Department through a collaborative scholarship with University of Georgia, Athens, USA. She completed a research project entitled 'Exploring IRES mediated discistrans for the phytoremediation of Mercury'. Currently, she is the Director of Pharmaceutical Bio-products Research Department, City of Scientific Research and Technology Applications, Egypt. Her main research interests are using plant tissue culture and genetic engineering techniques to conserve rare and endangered plant species as well as enhancement of the productivity of important pharmaceutical compounds from plants. She is working in several projects dealing with the production of antiviral, anticancer and antioxidant compounds from plants using in vitro cultures.

raoufaa@yahoo.com