Analysis of isozyme peroxidase under abiotic stress conditions in spinach A Review.

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Abstract*:*
Isoenzymes are multiple forms of enzyme which catalyze the same chemically reaction. Analyze of isoenzyme is promising tool in identification of plant growth parameters. Isozyme are important biomarkers to evaluate the genetic variations and biochemical properties of plant development. Electrophoretic technique are useful for characterization and identification of genetic marker which aim to distinguish or to confirm similarity between. Spinach (*Spinacia oleracea* L.) is a green plant flowering, most consumable in Asia. This belongs to family of Amaranthaceae. Polyacrylamide gel Electrophoresis (PAGE)is most commonly used to study the isozyme forms. Isozyme biomarks showed to be the mechanisms to understand the process of cellular differentiation and development of plants wich are suffering for biotic and abiotic stress conditions.

Keywords: Isoenzyme, Abiotic stress, Spinach, SDS-PAGE.

Introduction:

Sprouting seeds first meet unfavorable ecological conditions (water deficit, low and high salt, waterlogging) **(Bewley and Black., 1982)** thus leading to accumulation of damaging concentrations of reactive oxygen species (ROS)(Wang et al., 2004). In ideal focuses, ROS play a positive role in the normal plant development and in plant responses to environmental stresses. Under typical conditions the production and destruction of ROS is regulated well in cell metabolism but under stress conditions the formation of ROS exceeds the amount present under normal physiological conditions, thus creating the oxidative stress. Environmental stresses exert their effects directly or indirectly through the arrangement of ROS and ROS scavenging is a common response to most stresses (**Srivalli et al., 2003)**. Plants crop with stress by activation of the cell antioxidant system (**Gechev et al., 2002**). The ability of plant tissues to mobilize enzymatic defense against uncontrolled production of ROS may be of great importance for plant survival under stress conditions (**Andarson et al,. 1995**). It is well known that ABA levels increase in tissues subjected to dehydration stress **(Xiong, Zhu., 2001**). Under these conditions explicit qualities are communicated that can likewise be prompted in unstressed tissues by exogenously connected ABA. Therefore, the changes occurring in germinating wheat seeds in the presence of ABA can serve as a dehydration stress confirming standard. Chilling (0-15^{o} C) causes elevated levels of hydrogen peroxide in leaves of winter wheat and in maize seedlings (**Anderson et al., 1995**). Present study changes in seeds germinating in the presence of exogenously applied H_2O_2 were compared deals with those occurring in seeds germinated under low temperature (10^{o} C). Plant peroxides have been utilized as biochemical markers for different kinds of biotic and abiotic worries because of their significant job physiological procedures, similar to control of development by lignification, cross-connecting of gelatins and auxiliary proteins in cell divider, catabolism of auxins **(Gaspar et al., 1982)**. Catalases and superoxide dismutases are the most effective cancer prevention agent catalyst. The expression of specific catalase isoenzymes is important and critical against oxidative stress induced by a given environmental stress **(Scandalios, 1994)**. Low temperature stress causes the accumulation of H_2O_2, which in turn may function in increasing the activity of the CAT 1 and CAT 2 isoenzymes to prevent high accumulation of H_2O_2 and other ROS in plant cells **(Scandalios, 1984)**. Variable responses of SOD to dehydration stress have been reported in literature including decreased activity, lack of effect or increased activity **(Srivali, 2003)**depending on plant species, tissue and stage of development. The present review provide information to study the tissue-specific differences in the response of the antioxidant enzymes to stress.

Background:

Enzyme is a protein produced by all forms of living organisms which catalyze specific biochemical reaction. Many of enzymes are found in living cells where they act as catalysts for the multifaceted chemical reaction which occur in our daily routine. The enzymes which are existing in multiple molecular forms are defined as isoenzymes. Isozyme are different forms of enzymes that gain popularity because of their genetic properties and relationship between substrate and enzyme. In the establishment of isoenzymes involved structural variations that occur in proteins generally. Isoenzyme may be demonstrated by the position they have taken up after electrophoresis, isoelectric focusing, or column chromatography, as well as by difference in physical properties, chemical constitution and kinetics. Moreover one of the modes of action of isozyme, glucose 6 phosphate dehydrogenases. The isoenzyme markers are useful during the in vitro propagation to access the soma clonal variation and efficiency of micropropagation.

The Isoenzyme profiles of three enzyme system (peroxides, esterases, and acid phosphatase) of seven in vitro clones and native control were compared using discontinuous PAGE system **(T. Ivanova et. al., 2015)**.

The abiotic stress promoted by drought and salinity are limiting factor for productivity and crop growth. The number of countries in worldwide affected by this kind of stress is increasing and are mainly influenced by greenhouse emission **(Wang et. al., 2003).** The alternative approach to improve the tolerance of drought and salinity stress is to develop the resistant variety of crops . The knowledge of mechanism which mediate drought and salinity tolerance in plants have a role in development of new varieties and can also minimize the adverse effects. The commonly problematic abiotic stress are associated with drought which reduce approximately 90% of the crop production in most tropical countries **(Avramova et. al., 2015).** The prediction of climate change can became an alternative source to reduce yield loss and improve the rate of crop production, (**Zhan et. al., 2015)**. Salinity, are the second abiotic stress estimated to affect negatively 23% of cultivate lands on approximately 1.5 million ha per year in worldwide **(Eynard et al., 2006, (Tanji and Wallender., 2012).** The abiotic stress and oxidative stress are founded to induce the similar cellular damage during the development of crop . For example : during the stress induced by drought and salinity the plant undergo to osmotic stress and it may result in cell disruption, homeostasis and ion distribution (**Zhu., 2001**). Nevertheless similarity effects found in salinity and drought has been evaluated to understand the process that reduce yield and inhibit the growth (**Katerji et. al., 2004**).

Studies have been reported that the best approach to analyze the effect of salinity and drought stress is to evaluate each effect individual , which can be expressed as osmotic. A small works was completed to study how the salinity and water consumption affect the yield of crops. High salinity in the soil can became a limiting factor for development of most variety of crops (**Nagy and Galiba, 1995).**

Studies to understand the mechanisms involved in plant response during abiotic stress are the main importance in examination of salt content and water pressures allows retention of water system, and diminish a dirt dampness substance moisture content of 4% where it is kept for 10 more days. **(Ahmed et. al.,2013).** Accordingly studies made by **(Ahmed et. al.,2013)** the saturation of soil with periodic irrigations leads to delay in drought. Experiments done with matric potential showed that it is a good marker to measure the quantity of water which induce drought. This marker can became unknown or fluctuated during the experiment. Other molecular markers have showed to be a valuable source for accessing the plants genetic resources by adding the knowledge on distribution and the extent of genetic variation within and among species. Performance of isoenzymes activity of Peroxidases, Superoxide dismutase (SOD) can catalyze endosperm and roots of wheats seedling germinated under chronic stress conditions. Peroxidases enzyme are greater reference of biochemical markers for various types of biotic and abiotic stress. This enzyme have important role on control on plant development by lignifications cross connecting of gelatin and auxiliary structural proteins, cell divider catabolism of auxins **(Gaspar et. al., 1982)**.

Stress mediated by salt present a variable change on physiological and biochemical changes in plants. They are associated with a variety of expansive scope of development and formative procedures: they can induce cell division, dormancy ,germination of variety seeds, stimulation of growth, early development of flower buds, fruits, embryogenesis, plant morphogenesis and response to environmental stresses. Catalyze of Chloramphenicol acetyltransferase and Peroxidase are oxido-reductases enzymes that are involved in the molecular mechanisms of defense against reactive oxygen species. In spinach plant these two enzymes are commonly found sharing remarkable similarities in the reaction mechanism but respond differently to heme and Mg^{+2} activity. The peroxides enzyme act typically in catalyzation of oxidative reaction and biotic anxieties. Upgraded creation of oxygen free radicals is in charge of peroxidation of film lipids and the level of peroxidative harm of cells was constrained by the stronger activity of antioxidative substances (**Sreenivasulu et. al., 1999)**. Polyphenol oxidase (PPO) is isoenzyme composed of copper that enhance the hydroxylation of monophenols to o-diphenols and the oxidation of o-dihydroxyphenols to o-quinones utilizing molecular oxygen. These molecules are activated by electrophilic which covalently interconnected with variety of cellular molecules this kind of reaction result in unwanted product. Experiments conducted with (**Partington and Bolwell 1996**) found that PPO play a crucial role on food technology**.**

Genetic studies conducted in different legumes have proven that isozyme are good indicators to identify and characterize the variables affected during the abiotic stress. The combination of isozyme and DNA marker are very useful to differentiate morphological traits. Peroxidases are enzyme that have a been found to enhance the growth and differentiation plants. Peroxidases are most commonly in majority plant tissue and show variations in series of events of plant development. Although, variation in peroxides activity is associated with disease resistance, loss of pollen and seed viability, activity is particularly associated with auxins, besides growth and differentiation. Furthermore, Peroxidases have often served as a parameter for metabolic activity during growth alterations as they comprise of a family of several isozyme. Skins of mature tomato have been shown the presence of peroxidases isozyme **(Andrews et. al., 2001)**. The maturation of fruit are not connected with late appearing of isozymes may not linked with ethylene. To control the cross linking of fruit growth within the skin fruits, the use of Polyacrylamide Gel Electrophoresis are required to analyze the plant isoenzyme properties. Many studies use these two enzymes peroxidase and Poly Phenol Oxidase as biomarkers. These enzymes are also useful in studies conducted analyze different stages of plant growth and test it in different environmental conditions such as salinity, water deficiency, etc.

Conclusion

The present review , show that isoenzyme is potential molecular markers to evaluate abiotic stress in different variety of plants especially in spinach. Electrophoretic technique is more helpful on characterization and differentiation of isoenzyme. This tool also prove to be grater for biochemical analysis, as well as genetic markers using to find the similarities or differences among species. However, the use of isozymes in combination with DNA markers is still considered to be very useful and important in distinguishing morphological traits. The Peroxidases are enzymes which have a been showed to improve the growth rate and development of higher plants Peroxidases are present in almost every plant tissues and their levels are distributed according to plant developmental events. Peroxidase isozyme have a capability to induce and increase shoot bud differentiation. Because of that we concluded that their corelate with each other. Studies using a isozyme as indicators are helpful tools in understanding of the mechanisms involved in cellular differentiation, plant development. Results for many works suggest that PO and PPO are potent biomarkers to study plant growth development parameters by SDS -PAGE techniques.

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