Enzymatic activity profiles at different stages of the development of bulbils from "Dougou-won" and "Won-kpia," two varieties *of Dioscorea bulbifera*

Abstract

Two varieties of *Dioscorea bulbifera* (Dougou-won and Won-kpia) were grown to study the enzymatic profile of bulbils during their growth. For each variety, bulbils aged between 1 and 6 months were harvested. After being washed, peeled and cut into strips, the samples were ground in a 0.9% NaCl saline solution. The mixture obtained was centrifuged and the supernatant collected constituted the crude enzyme extract. The experimental conditions consisted of a reaction medium comprising a buffer solution, the enzyme extract and synthetic or natural substrates. The results showed that the bulbils of "Dougou-won" have high levels of polyphenol oxidase (PPO), peroxidase (POD), acid phosphatase and α-amylase, while the bulbils of "Won-kpia" showed high levels of α-D-mannosidase, β-fucosidase and inulase during growth. The study highlighted the enzymatic potential of bulbils of the "Dougou-won" and "Won-kpia" varieties, which could be a valuable source of enzymes for biotechnology and other industrial.

Keyword: Bulbils, "Dougou-won", "Won-kpia", Enzymatic activity

1. INTRODUCTION

Dioscorea bulbifera, also known as aerial tubers, is a type of yam that is native to tropical Africa and Southeast Asia. It belongs to the Dioscoreaceae family and the Dioscorea genus, which contains 603 species [1, 2]. A plant is a climbing stem that, to grow vertically, relies on a stake around which it winds itself. Winding takes place on the sinister side (counter-clockwise), and the rapid growth of the stem is reflected in the lengthening of the internodes. Alternate secondary branches develop from the stems. The simple, very broad, alternate leaves are light or dark green. They have a long petiole with stipules, a cordate, and a palmatinervate blade. Small, ovoid, or spherical swellings with varying degrees of angularity develop very early in the leaf axils. These small organs, or bulbils, grow and reach approximately 10 cm in diameter following the accumulation of reserves. On stems, bulbils are very abundant and variable in shape, often polyhedral and sometimes rounded, grey or brown [3]. Underground tubers may be present or absent.

In Côte d'Ivoire, the Bété ethnic group, who live in forested areas, classify *Dioscorea bulbifera* bulbils into three groups. The first group is called 'Kou-won' (or genie bulbils) represents the no edible wild species. The second group, called 'Dougou-won', is characterised by large bulbils (weighing up to 300 grams) with grey skin and yellow flesh. The third group, called 'Won-kpia', is characterised by medium-sized bulbils (up to 60 grams) with brown skin and purplish flesh, with brown skin and purplish flesh [4]. Both "Dougou-won" and "Won-kpia" are bulbils with a succulent taste that is highly appreciated by the local population. To consume them, bulbils are boiled with their skin on, and then the skin is removed and eaten.

Dioscorea bulbifera bulbils have significant ethnomedicinal value. Indeed, several studies have been carried out on its various medicinal uses. In traditional medicine, they are used for the treatment of a variety of ailments such as syphilis, skin infections, throat infections, cough, epistaxis, goitre, haemoptysis, pharyngitis, and to remove dandruff, dysentery, ulcers, and haemorrhoids [5]. Additionally, due to their high diosgenin (saponin) content, bulbils are a natural source for the industrial production of a number of steroid hormones, such as those used in hormonal contraception [6, 7]. Pharmaceutical industry also uses them to manufacture anti-inflammatories, metabolic stimulants, and antidepressants [8, 9]. These biomolecules are produced by enzymes in the bulbils.

Enzymes are biological molecules that play a crucial role in various cellular processes, including metabolism, growth, and development. In plants, enzymes are involved in the breakdown of complex molecules into simpler compounds, which provides energy and building blocks for growth and development. In the context of bulbils, enzymes are likely to play a key role in the formation and maturation of these structures. Previous studies have reported on the enzymatic activities present in different plant tissues, including leaves and tubers [10]. However, there is limited information available on the enzymatic profiles of bulbils of *D. bulbifera*, particularly at different stages of growth. Understanding the changes in enzymatic activity that occur during the growth and development of bulbils is essential for optimizing their cultivation and improving their nutritional value.

In this study, we aimed to investigate the enzymatic profiles of bulbils from "Dougou-won" and "Won-kpia", two varieties of *D. bulbifera* cultivated in Ivory Coast during the growth stage. Specifically, we examined the activities of some enzymes involved in various cellular processes, including carbohydrate metabolism, protein degradation, and defence responses. Our results provide new insights into the enzymatic profiles of these two varieties and highlight the potential applications of these enzymes and understanding the role in plant development and

maturation that may allow for the development of new strategies for improving crop yields or enhancing plant resistance to diseases.

2. MATERIALS AND METHODS

2.1. Materials

The bulbils of the "Dougou-won" and "Won-kpia" varieties (*Dioscorea bulbifera*) used in this study were cultivated during the suitable cropping season at the experimental farm of the University Nangui Abrogoua (Abidjan-Côte d'Ivoire, at a latitude of 5º23' North, longitude of 4º00' West, and an altitude of 7 meters) in May 2020. Each month (from July to December), bulbils from both varieties were harvested based on their age, ranging from one to six months, and then transported to the laboratory for analysis. All chemicals and reagents used were of analytical grade and sourced from Sigma Chemical Co. (St. Louis, MO).

2.2 Methods

2.2.1. Enzyme Extraction

The enzyme extraction was conducted according the method described by [11. Bulbils at various stages of growth from each cultivar were cleaned by rinsing with tap water followed by distilled water. Cleaned bulbils were then peeled, sliced and processed into a slide using a stainless steel knife. All cutting operations were performed at ambient temperature (27-30°C). Fifty grams of sliced bulbils were ground in a MOULINEX grinder with 20 mL of 0.9% (w/v) NaCl solution. Resulting homogenate was subjected to sonication using a TRANSSONIC T420 device for 10 minutes. Sonicated homogenate was then centrifuged at 6,000 rpm for 15 minutes at 4°C. Resulting supernatant was collected and used as the crude extract. Extract was stored at 21°C.

2.2.2. Polyphenol oxidase (PPO) activity assay

Polyphenol oxidase (PPO) activity was measured according to the method proposed by [12]. In a test tube, were successively added 2.85 ml of 0.2 mM (pH 7) phosphate buffer, 50 µl of catechol (60 mM) as a substrate and 100 µl of enzymatic crude extract. The mixture was maintained at 25°C and the change in absorbance was read over 3 min at 420 nm using a Jenway 6,505 UV/Vis spectrophotometer.

2.2.3. Peroxidase (POD) activity assay

Enzyme activity was measured using a methylene blue substrate assay [13]. Assay mixture consisted of 2.2 mL of diluted supernatant, 0.1 mL of 1.2 mM methylene blue, and 0.6 mL of

0.5 M sodium tartrate buffer (pH 4.0). Reaction was initiated by adding 0.1 mL of 2.7 mM hydrogen peroxide. Enzyme activity was monitored by tracking the decrease in absorbance at 664 nm, which corresponds to the conversion of methylene blue to Azure C.

2.2.4. *p*NP-phosphatase, *p*NP-(α, β)-glycosidase activity assays

Enzyme activity assays were performed using the pNP-phosphatase and pNP-(α,β)-glycosidase assays. Reactions were conducted under standard conditions, where 250 µl of an enzyme assay mixture containing 75 µl of substrate constitute of either pNP-(α,β)-D-glycoside (for (α,β)-glycosidase) or pNP-phosphate (for phosphatase) (5 mM) in 100 mM sodium acetate buffer (pH 5.0) with 50 µl enzyme solution was incubated at 37°C for 10 minutes. A reference cell containing all reactants except the enzyme was also prepared. Reaction was terminated by adding 2 ml of sodium carbonate (2%, w/v), and the absorbance of the assay solution was measured at 410 nm using a spectrophotometer GENESYS 5.

2.2.5. Polysaccharidase activity assays

Polysaccharidase activity were assayed by the 3.5-dinitrosalicylic acid (DNS) procedure [14], using 1% (w/v) polysaccharide (carboxymethyl cellulose, sucrose, inulin, and starch) as substrate. Enzyme (50 µl) was incubated for 30 min at 37°C with 170 µl sodium acetate buffer (100 mM, pH 5.0) and 80 µl polysaccharide. Reaction was stopped by adding 300 µl DNS solution and heating for 5 min in boiling water bath. Absorbance was read at 540 nm after cooling on ice for 5 min.

2.2.6. Protein assays

Protein concentrations were determined using the Lowry method [15] with bovine serum albumin as the standard reference.

2.2.7. Enzymatic activity expression

Enzymatic activity was expressed as units per mg of protein (UI/mg of protein). One unit of enzyme activity is defined as the amount of enzyme capable of releasing one µmol of product per minute under the defined reaction conditions.

2.2.8. Statistical analysis

All analyses reported in this study were carried out in triplicates. Mean value and standard deviation were calculated. Statistical significance was established using Analysis of Variance (ANOVA) models to estimate the effect of growth stage enzymatic activities in bulbils from

"Dougou-won" and "Won-kpia" variety. Means were separated according to Duncan's multiple range analysis (P≤0.05), with the help of the software STATISCA 7.1 (Stat Soft Inc, Tulsa USA Headquarters).

3. RESULTS AND DISCUSSION

3.1. Antioxidant enzymes: Polyphenoloxidase (PPO) and peroxidase (POD) activities

The enzymatic activities of PPO and POD in the bulbils of "Dougou-won" and "Won-kpia" are presented in Figures 3 and 4. Values of enzymatic activities from the first to sixth month were 105.40 ± 2.5 - 130.40 ± 1.10 UI/mg of protein and 38.25 ± 1.10 - 70.12 ± 2.41 UI/mg of protein for "Dougou-won," while those of "Won-kpia" were 80.20 ± 2.41 - 111.80 ± 2.10 UI/mg of protein and 22.10 ± 1.12 - 46.25 ± 2.51 UI/mg of protein, respectively. These results show that both PPO and POD activities were detected at the beginning of bulbils tuberization and increased during their development. Enzymatic activities of bulbils from "Dougou-won" were higher than those of "Won-kpia," with PPO activities being higher than POD activities in both varieties. This study highlights the significance of PPO and POD in the metabolic processes of tubers and their potential roles in fruit growth and ripening and plant defence against pathogens [16, 17, 18, 19, 20, 21]. In addition, polyphenol oxidases have been identified as being responsible for the enzymatic browning of plant products, a phenomenon that significantly alters their appearance and texture [22, 23, 24].

3.2. Acid phosphatase activity

During the development of bulbils from both D. bulbifera varieties, "Dougou-won" and "Won-kpia," acid phosphatase activity was significantly increased (Fig. 5). Enzymatic activity in bulbils of the "Dougou-won" variety increased over time, from 21.51±2.40 UI/mg of protein $x10^{-2}$ at the first month to 69.26±2.93 UI/mg of protein $x10^{-2}$ at the sixth month, while in "Won-kpia" bulbils, the activity increased from 26.44±1.30 UI/mg of protein $x10^{-2}$ at the first month to 65.01±2.85 UI/mg of protein $x10^{-2}$ at the sixth month. Notably, acid phosphatase activity was significantly higher in bulbils from "Dougou-won" compared to those of "Won-kpia." This intense activity is attributed to the crucial role of acid phosphatase in regulating various cellular processes, including energy transfer, photosynthesis, respiration, metabolic reactions, signalling, and structural components of biomolecules [25]. Moreover, phosphatases play a key role in starch metabolism by dephosphorylating starch phosphorylase and modulating starch synthesis and degradation during bulbil development [10, 25, 26]. Additionally, phosphatases

can remove phosphate groups from proteins, influencing their activity, localisation, and stability, which is essential for regulating enzyme activity and hormone signalling during bulbil development.

3.3. α-D-Glycosidase activities

During the development of bulbils from cultivars “Dougou-won” and "Won-kpia," various α-D-glycosidases were detected, including α-D-mannosidase, α-D-glucosidase, α-D-xylosidase, α-D-fucosidase, and α-D-galactosidase (figures 6 and 7). Notably, α-D-mannosidase exhibited the highest activity levels in both varieties. Specifically, at the first and sixth months of growth, the proportion of enzymatic activities in bulbils of the “Dougou-won” variety was: α-D-mannosidase (48–39 UI/mg of protein $x10^{-2}$), α-D-fucosidase (38–17 UI/mg of protein $x10^{-2}$), α-D-glucosidase (9–20%), α-D-xylosidase (4–22 UI/mg of protein $x10^{-2}$), and α-D-galactosidase (4–2 UI/mg of protein $x10^{-2}$). In contrast, the proportion of enzymatic activities in bulbils from the “Won-kpia” variety was: α-D-mannosidase (67–68 UI/mg of protein $x10^{-2}$), α-D-glucosidase (11–15 UI/mg of protein $x10^{-2}$), α-D-fucosidase (4–8 UI/mg of protein $x10^{-2}$), α-D-xylosidase (4–22 UI/mg of protein $x10^{-2}$), and α-D-galactosidase (4–2 UI/mg of protein $x10^{-2}$). These results indicate significant differences in the activity profiles between the two cultivars. Results suggest that α-D-mannosidase plays a crucial role in the development of bulbils from both cultivars. High activity levels of α-D-mannosidase in both varieties indicate that this enzyme is essential for the degradation of substrates containing α-D-mannose residues. This is particularly important during seed germination, growth, and fruit ripening, as reported by [27]. The significant differences in the activity profiles between the two cultivars may be related to their different genetic backgrounds and environmental conditions. Higher activity levels of α-D-mannosidase in the “Won-kpia” variety may be an adaptation to its specific growing conditions, allowing it to optimise its growth and development. In contrast, the “Dougou-won” variety may have a lower activity level of α-D-mannosidase due to its different genetic makeup or environmental conditions. The study also highlights the potential importance of α-D-mannosidases in plant development and maturation, as reported in previous research [28, 29]. By removing α-D-mannose residues from glycolipids and glycoproteins, these enzymes may facilitate the mobilisation of proteins and lipids, which is crucial for plant growth and development. This suggests that α-D-mannosidases may be a key player in regulating plant metabolism and development. Furthermore, the study provides insight into the potential applications of plant glycosidase in agriculture and biotechnology.

3.4. β-Glycosidase activities

β-glucosidase, β-xylisidase, β-fucosidase, and β-galactosidase activities in the bulbils of "Dougou-won" and "Won-kpia" during growth were presented by figures 8 and 9, respectively. Results showed that the proportion of enzymatic activities in bulbils of the "Dougou-won" variety at the first and sixth months was predominantly composed of β-D-glucosidase (76-34 UI/mg of protein $x10^{-2}$), followed by β-D-xylosidase (10-12 UI/mg of protein $x10^{-2}$), β-D-galactosidase (12-28 UI/mg of protein $x10^{-2}$), and β-D-fucosidase (2-26 UI/mg of protein $x10^{-2}$). In contrast, the proportions of enzymatic activities in bulbils of the "Won-kpia" variety were: β-D-glucosidase (41-25 UI/mg of protein $x10^{-2}$), β-D-xylosidase (12–12 UI/mg of protein $x10^{-2}$), β-D-fucosidase (36–52 UI/mg of protein $x10^{-2}$), and β-D-galactosidase (11 UI/mg of protein $x10^{-2}$). Notably, the β-D-glucosidase activity was higher than the other activities in the "Dougou-won" variety, while in the "Won-kpia" variety, the β-D-glucosidase activity had the highest until the fourth month but was surpassed by the β-D-fucosidase activity at the sixth month. Results suggest that the two varieties of plants have distinct metabolic profiles, which may be influenced by their genetic makeup or environmental conditions. The dominance of β-glucosidase activity in the "Dougou-won" variety may indicate a preference for glucose-based metabolic pathways, whereas the shift to β-fucosidase activity in the "Won-kpia" variety may indicate a greater reliance on fucose-based pathways. Differences in enzyme activity patterns between the two varieties may have implications for their tolerance to different environmental conditions. For example, plants with higher levels of β-glucosidase activity may be more tolerant of drought or high temperatures, as glucose is a key molecule involved in stress responses [30]. In contrast, plants with higher levels of β-fucosidase activity may be more tolerant of acidic or nutrient-poor conditions. High levels of β-glucosidase activity in bulbils of the "Dougou-won" variety could be exploited for the production of biofuels or bioproducts from glucose-based biomass. β-glucosidase could also be used in the environment, particularly in the degradation of glucosides present in soils and water. Similarly, the high level of β-fucosidase activity in the "Won-kpia" variety could open up opportunities, including the production of biofuels and agricultural applications to improve plant resistance to disease and insects by modifying the glycosidic structures of their cell walls.

3.5. Polysaccharidase activities: Invertase, inulase, α-amylase and cellulase activities

Results show that the invertase, inulase, α-amylase, and cellulase activity profiles of the "Dougou-won" variety change over time, with a lower activity at the first three months and a subsequent increase in α-amylase, invertase, and cellulase activities up to the sixth month (Fig.

10). In contrast, the “Won-kpia” variety exhibits a progressive increase in inulase activity until the fifth month, followed by a decrease (Fig. 11). The proportion of enzymatic activities in both varieties reveals that α-amylase is the dominant enzyme in bulbils from both varieties at the maturation stage, whereas inulase activity is highest in the “Won-kpia” variety until the fifth month. The presence of these enzymes suggests the presence of starch, sucrose, inulin, and cellulose in these tuber varieties [31, 32]. Thanks to their high level of α-amylase, bulbils from “Dougou-won” offer a promising new source of this enzyme, which has significant potential in biotechnology for the production of biofuels and biopesticides and in industry for the manufacture of bioplastics and the treatment of wastewater and organic waste. Also, due to its high inulase activity, bulbils from “Won-kpia” are a new source of this enzyme, which has numerous applications in biotechnology and industry.

CONCLUSION

This study showed that bulbils of the “Dougou-won” and “Won-kpia” varieties of *Dioscorea bulbifera* develop intense enzymatic activities during their growth. Polyphenol oxidase (PPO), peroxidase (POD) and acid phosphatase are the best expressed activities. In addition, “Dougou-won” bulbils have high levels of PPO, POD, acid phosphatase and α-amylase, while “Won-kpia” bulbils have high levels of α-D-mannosidase, β-fucosidase and inulase. The study highlighted the enzymatic potential of bulbils of the “Dougou-won” and “Won-kpia” varieties, which could be a valuable source of enzymes for biotechnology and other industrial applications.

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Fig 1: Photos of Bulbils from "Dougou-won "variety

Fig 2: Bulbils from "Won-kpia" variety

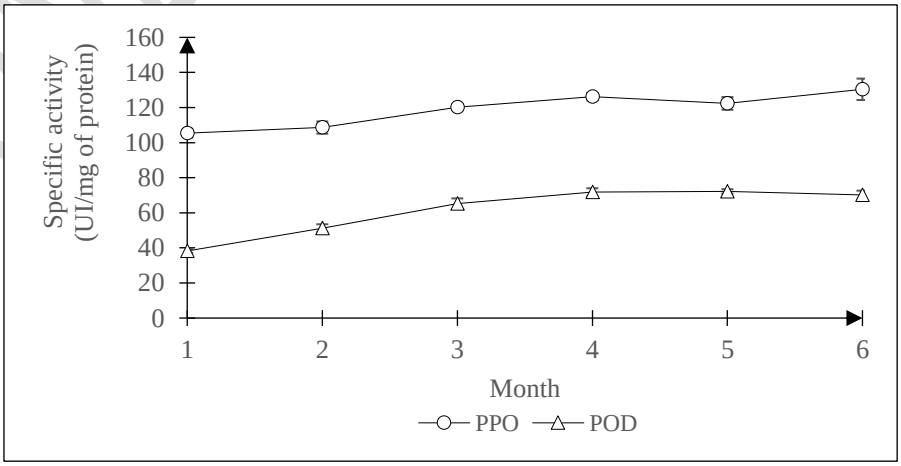

Fig 3: PPO and POD activities in bulbils from "Dougou-won" variety during the growth.

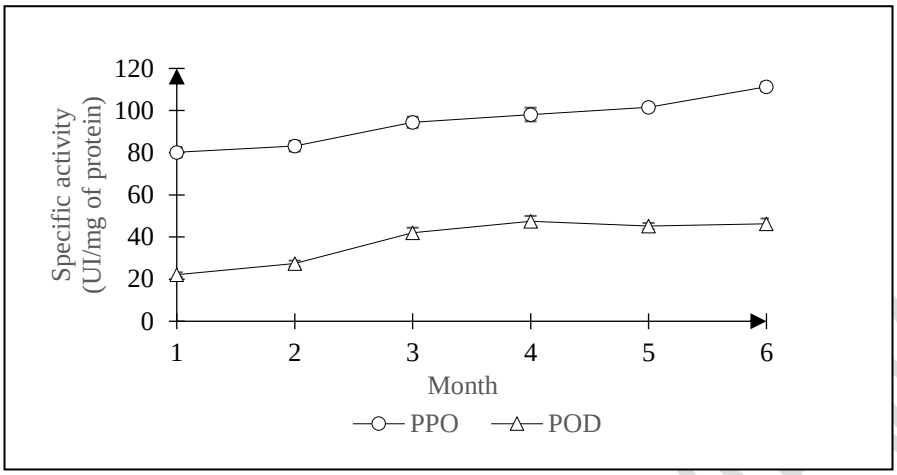

Fig 4: PPO and POD activities in bulbils from "Won-kpia" variety during the growth.

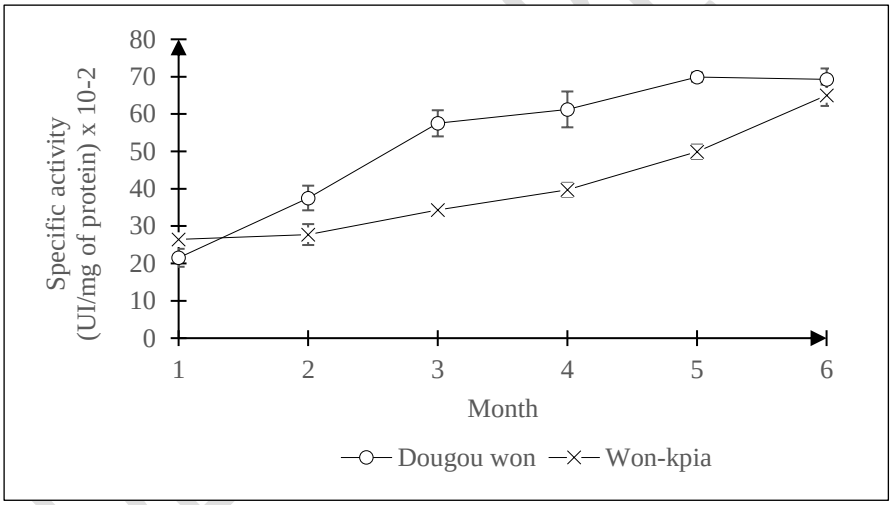

Fig 5: Acid Phosphatase activities in bulbils from "Dougou-won" and "Won-kpia" varieties during the growth.

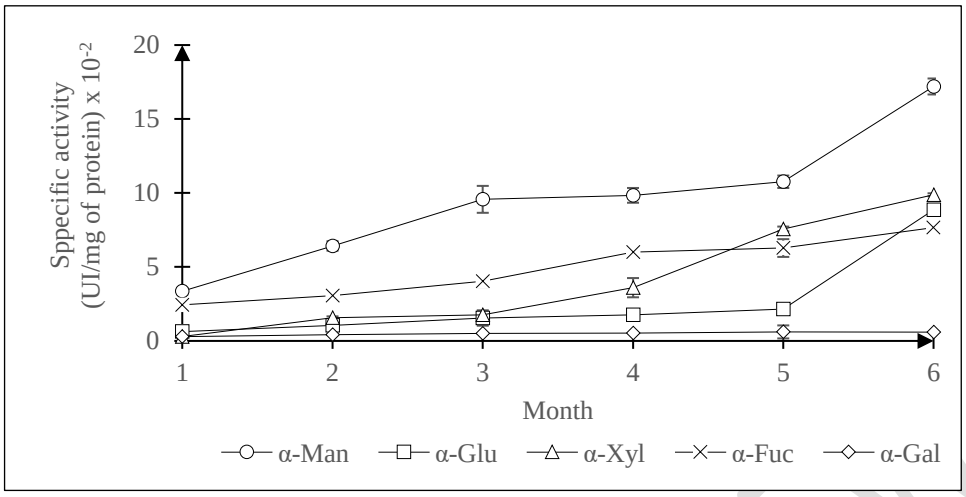

Fig 6: α-mannosidase, α-glucosidase, α-xylosidase, α-fucosidase and α-galactosidase activities in bulbils from" Dougou-won" variety during the growth.

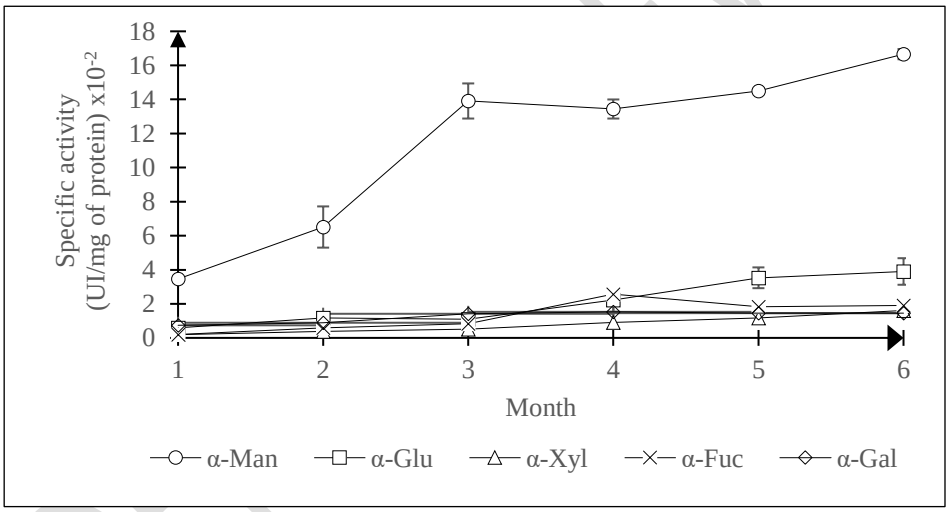

Fig 7: α-glucosidase, α-xylosidase, α-fucosidase, α-galactosidase, and α-arabinosidase activities in bulbils from "Won-kpia" variety during the growth.

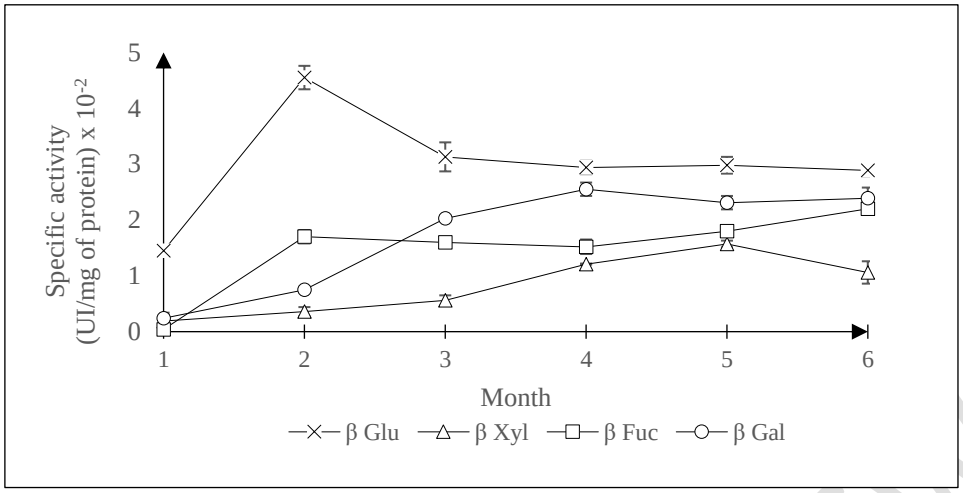

Fig 8: β-glucoosidase, β-xylosidase, β-fucosidase and β-galactosidase activities in bulbils from "Dougou-won" variety during the growth.

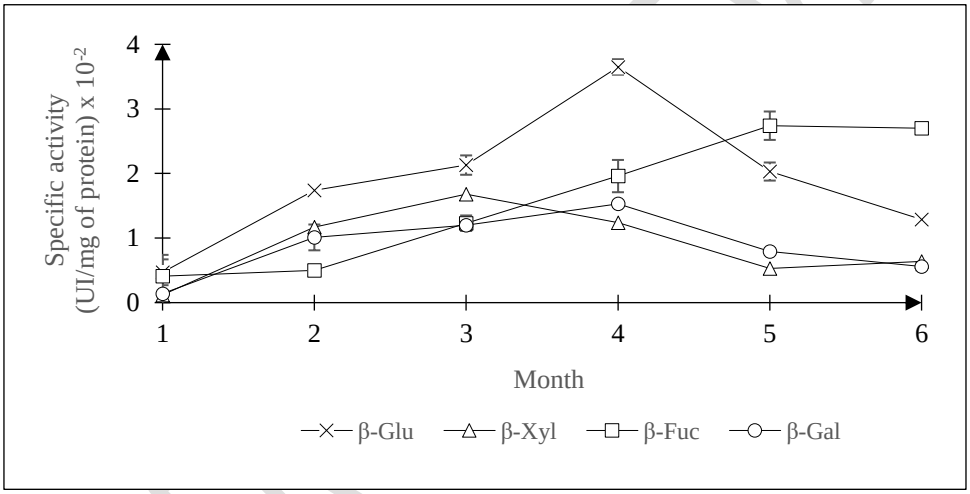

Fig 9: β-glucoosidase, β-xylosidase, β-fucosidase and β-galactosidase activities in bulbils from "Won-kpia" variety during the growth.

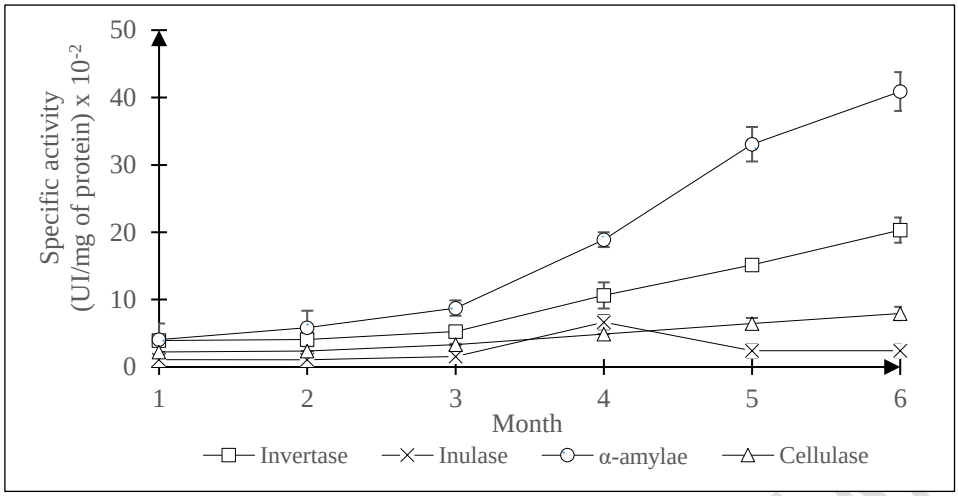

Fig 10: Invertase, inulase, α-amylase and cellulase activities in bulbils from "Dougou-won" variety during the growth.

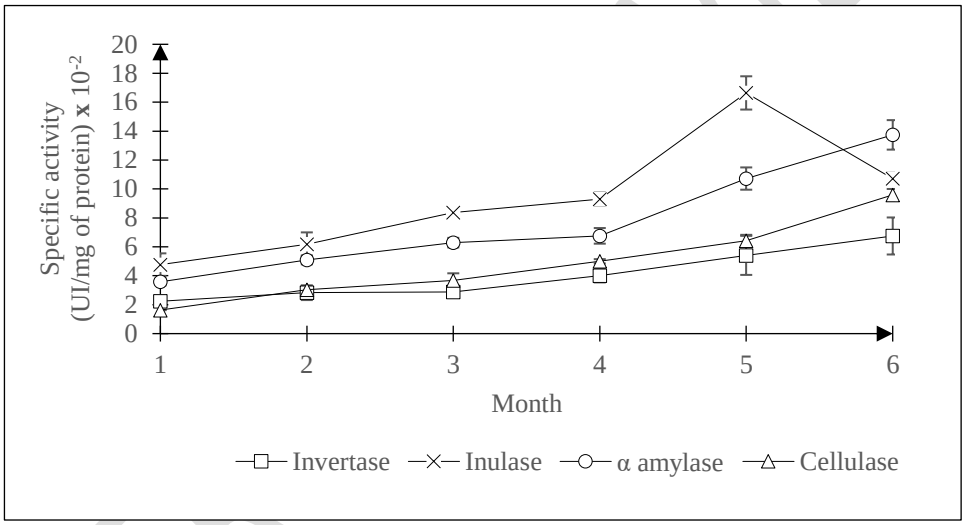

Fig 11: Invertase, inulase, α-amylase and cellulase activities in bulbils from "Won-kpia" variety during the growth.