

## Enzymatic activity profiles at different stage of development of bulbils from “Dougou-won” and “Won-kpia”, two varieties of *Dioscorea bulbifera*

### Abstract

This study investigates the enzymatic activities of polyphenoloxidase (PPO), peroxidase (POD), acid phosphatase,  $\alpha$ -D-glycosidase,  $\beta$ -glycosidase, invertase,  $\alpha$ -amylase, inulase and cellulase in bulbils of “Dougou-won” and “Won-kpia”, two varieties of *Dioscorea bulbifera*. Results show that PPO and POD activities are detected at the beginning of bulbil tuberization and increase during development, with “Dougou-won” exhibiting higher enzymatic activities than “Won-kpia”. Acid phosphatase activity is significantly increased in both varieties, while  $\alpha$ -D-mannosidase plays a crucial role in the development of bulbils from both cultivars. Study also reveals distinct metabolic profiles between the two varieties, with differences in enzyme activity patterns potentially influencing their tolerance to different environmental conditions. Findings highlight the importance of these enzymes in regulating plant metabolism and development, and their potential applications in agriculture and biotechnology and industry.

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 South-East Asia. It belongs to the Dioscoreaceae family and the *Dioscorea* genus, which contains 603 species [1, 2]. Plant is a climbing stem which, in order 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 in colour. They have a long petiole with stipules and a cordate, palmatinervate blade. In the leaf axils, small, ovoid or spherical swellings with varying degrees of angularity develop very early on. These small organs, or bulbils grow and reach approximately 10 cm in diameter following the accumulation of reserves. On stems, bulbils are very abundant with variable in shape, often polyhedral and sometimes rounded, grey or brown in colour [3]. Underground tubers may be present or absent.

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The purpose, place of study, methods of work, results and conclusions must be taken into account in brief.

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**Commented [DHN4]:** *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 Ivory Coast, Bétés ethnic group, who live in forest areas, categorize *Dioscorea bulbifera* into three groups. First group is called “Kou-won”, which refers to the wild species. Term “Kou-won” is composed of two words: “Kou” means genie, and “won” means bulbils. This species grows naturally in clearings, forests, and under coffee trees. It is characterized by exuberant vegetation and leaves that are often smaller than those of cultivated varieties. The bulbils produced by this species are of varying sizes and are not edible. Second group is called “Dougou-won”, which has very broad, light-green leaves. This variety produces large bulbils that can weigh over 300g, with gray skin and yellow flesh. Third group is called “Won-kpia”, which has dark-green leaves and produces medium-sized bulbils of up to 60 grams, 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

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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.

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## 2. MATERIALS AND METHODS

### 2.1. Materials

At the University of Nangui Abrogoua's experimental farm in Abidjan, Ivory Coast, West Africa, two varieties of *Dioscorea bulbifera*, namely "Dougou-won" and "Won-kpia", were cultivated in 2020. A field experiment was conducted, where bulbils of different ages (1, 2, 3, 4, 5, and 6 months) were harvested and transported to the laboratory for analysis. All chemicals and reagents used were of analytical grade and sourced from Sigma Chemical Co. (St. Louis, MO).

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### 2.2 Methods

#### 2.2.1. Enzyme Extraction

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.

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#### 2.2.2. Polyphenol oxidase (PPO) activity assay

Polyphenol oxidase (PPO) activity was measured according to the method proposed by [11]. 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. 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.

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#### 2.2.3. Peroxidase (POD) activity assay

Enzyme activity was measured using a methylene blue substrate assay [12]. 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. pNP-phosphatase, pNP-( $\alpha$ , $\beta$ )-glycosidase activity assays**

Enzyme activity assays were performed using the pNP-phosphatase and pNP-( $\alpha$ , $\beta$ )-glycosidase assays. Reactions were conducted under standard conditions, where 250  $\mu$ l of an enzyme assay mixture containing 75  $\mu$ l of substrate constitute of either pNP-( $\alpha$ , $\beta$ )-D-glycoside (for ( $\alpha$ , $\beta$ )-glycosidase) or pNP-phosphate (for phosphatase) (5 mM) in 100 mM sodium acetate buffer (pH 5.0) with 50  $\mu$ l 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 [13], using 1% (w/v) polysaccharide (carboxymethyl cellulose, sucrose, inulin, and starch) as substrate. Enzyme (50  $\mu$ l) was incubated for 30 min at 37°C with 170  $\mu$ l sodium acetate buffer (100 mM, pH 5.0) and 80  $\mu$ l polysaccharide. Reaction was stopped by adding 300  $\mu$ l 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 [14] 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  $\mu$ mol 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 ( $P \leq 0.05$ ), 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

Enzymatic activities of PPO and POD in 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  $105.40 \pm 2.5 - 130.40 \pm 1.10$  UI/mg of protein and  $38.25 \pm 1.10 - 70.12 \pm 2.41$  UI/mg of protein for “Dougou-won”, while those of “Won-kpia” were  $80.20 \pm 2.41 - 111.80 \pm 2.10$  UI/mg of protein and  $22.10 \pm 1.12 - 46.25 \pm 2.51$  UI/mg of protein, respectively. These results show that both PPO and POD activities were detected at the beginning of bulbil tuberization and increased during 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 [15, 16, 17, 18, 19, 20]. 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 [21, 22, 23].

#### 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 “Dougou-won” variety increased over time, from  $21.51 \pm 2.40$  UI/mg of protein  $\times 10^{-2}$  at the first month to  $69.26 \pm 2.93$  UI/mg of protein  $\times 10^{-2}$  at the sixth month, while in “Won-kpia” bulbils, the activity increased from  $26.44 \pm 1.30$  UI/mg of protein  $\times 10^{-2}$  at the first month to  $65.01 \pm 2.85$  UI/mg of protein  $\times 10^{-2}$  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,

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signalling, and structural components of biomolecules [24]. Moreover, phosphatases play a key role in starch metabolism by dephosphorylating starch phosphorylase, modulating starch synthesis and degradation during bulbil development [10, 24, 25]. Additionally, phosphatases can remove phosphate groups from proteins, influencing their activity, localization, and stability, which is essential for regulating enzyme activity and hormone signalling during bulbil development.

### 3.3. $\alpha$ -D-Glycosidase activities

During the development of bulbils from cultivars “Dougou-won” and “Won-kpia”, various  $\alpha$ -D-glycosidases were detected, including  $\alpha$ -D-mannosidase,  $\alpha$ -D-glucosidase,  $\alpha$ -D-xylosidase,  $\alpha$ -D-fucosidase, and  $\alpha$ -D-galactosidase (figures 6 & 7). Notably,  $\alpha$ -D-mannosidase exhibited the highest activity levels in both varieties. Specifically, at the first and sixth month of growth, the proportion of enzymatic activities in bulbils of “Dougou-won” variety was:  $\alpha$ -D-mannosidase (48-39%),  $\alpha$ -D-fucosidase (38-17%),  $\alpha$ -D-glucosidase (9-20%),  $\alpha$ -D-xylosidase (4-22%), and  $\alpha$ -D-galactosidase (4-2%). In contrast, the proportion of enzymatic activities in bulbils from “Won-kpia” variety was:  $\alpha$ -D-mannosidase (67-68%),  $\alpha$ -D-glucosidase (11-15%),  $\alpha$ -D-fucosidase (4-8%),  $\alpha$ -D-xylosidase (4-22%), and  $\alpha$ -D-galactosidase (4-2%). These results indicate significant differences in the activity profiles between the two cultivars. Results suggest that  $\alpha$ -D-mannosidase plays a crucial role in the development of bulbils from both cultivars. High activity levels of  $\alpha$ -D-mannosidase in both varieties indicate that this enzyme is essential for the degradation of substrates containing  $\alpha$ -D-mannose residues. This is particularly important during seed germination, growth, and fruit ripening, as reported by [26]. 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  $\alpha$ -D-mannosidase in “Won-kpia” variety may be an adaptation to its specific growing conditions, allowing it to optimize its growth and development. In contrast, “Dougou-won” variety may have a lower activity level of  $\alpha$ -D-mannosidase due to its different genetic makeup or environmental conditions. Study also highlights the potential importance of  $\alpha$ -D-mannosidases in plant development and maturation as reported in previous research [27, 28]. By removing  $\alpha$ -D-mannose residues from glycolipids and glycoproteins, these enzymes may facilitate the mobilization of proteins and lipids, which is crucial for plant growth and development. This suggests that  $\alpha$ -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.

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### 3.4. $\beta$ -Glycosidase activities

$\beta$ -glucosidase,  $\beta$ -xylosidase,  $\beta$ -fucosidase and  $\beta$ -galactosidase activities in bulbils of “Dougou-won” and “Won-kpia” during the growth were presented by figures 8 and 9 respectively. Results showed that the proportion of enzymatic activities in bulbils of “Dougou-won” variety at first and sixth month was predominantly composed of  $\beta$ -D-glucosidase (76-34%), followed by  $\beta$ -D-xylosidase (10-12%),  $\beta$ -D-galactosidase (12-28%), and  $\beta$ -D-fucosidase (2-26%). In contrast, the proportions of enzymatic activities in bulbils of “Won-kpia” variety were:  $\beta$ -D-glucosidase (41-25%),  $\beta$ -D-xylosidase (12-12%),  $\beta$ -D-fucosidase (36-52%), and  $\beta$ -D-galactosidase (11%). Notably, the  $\beta$ -D-glucosidase activity was higher than the other activities in “Dougou-won” variety, while in “Won-kpia” variety, the  $\beta$ -D-glucosidase activity had the highest until the fourth month, but was surpassed by the  $\beta$ -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. Dominance of  $\beta$ -glucosidase activity in “Dougou-won” variety may indicate a preference for glucose-based metabolic pathways, whereas the shift to  $\beta$ -fucosidase activity in “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  $\beta$ -glucosidase activity may be more tolerant of drought or high temperatures, as glucose is a key molecule involved in stress responses [29]. In contrast, plants with higher levels of  $\beta$ -fucosidase activity may be more tolerant of acidic or nutrient-poor conditions. High levels of  $\beta$ -glucosidase activity in bulbils of the “Dougou-won” variety could be exploited for the production of biofuels or bioproducts from glucose-based biomass.  $\beta$ -glucosidase could also be used in the environment, particularly in the degradation of glucosides present in soils and water. Similarly, the high level of  $\beta$ -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, $\alpha$ -amylase and cellulase activities

Results show that the Invertase, inulase,  $\alpha$ -amylase and cellulase activities profile of “Dougou-won” variety changes over time, with a lower activity at the first three months and a subsequent increase in  $\alpha$ -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

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month, followed by a decrease (fig 11). Proportion of enzymatic activities in both varieties reveals that  $\alpha$ -amylase is the dominant enzyme in bulbils from both varieties at the maturation stage, whereas inulase activity is highest in “Won-kpia” variety until the fifth month. Presence of these enzymes suggests the presence of starch, sucrose, inulin, and cellulose in these tuber varieties [30, 31]. Thanks to their high level of  $\alpha$ -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.

#### 4. CONCLUSION

In conclusion, this study has investigated the enzymatic activities of antioxidant enzymes, acid phosphatase,  $\alpha$ -glycosidase, and  $\beta$ -glycosidase in bulbils from two varieties of *D. bulbifera*, “Dougou-won” and “Won-kpia”. Results show that both varieties exhibit significant changes in enzymatic activities during the development of bulbils. Specifically, “Dougou-won” exhibits higher levels of polyphenol oxidase (PPO), peroxidase (POD), and  $\alpha$ -amylase activities, while “Won-kpia” exhibits higher levels of  $\alpha$ -D-mannosidase,  $\beta$ -fucosidase, and inulase activities. Study highlights the importance of these enzymes in regulating various cellular processes, including energy transfer, photosynthesis, respiration, metabolic reactions, signaling, and structural components of biomolecules. Furthermore, the results suggest that the two varieties have distinct metabolic profiles, which may be influenced by their genetic makeup or environmental conditions. Moreover, the bulbils from “Dougou-won” and “Won-kpia” have potential as a source of enzymes that are useful in biotechnology and industry applications.

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**Commented [DHN22]:** Results show that the invertase, inulase,  $\alpha$ -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  $\alpha$ -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  $\alpha$ -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 [30, 31]. Thanks to their high level of  $\alpha$ -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.

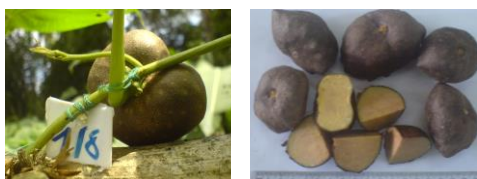
**Commented [DHN23]:** shorten it long

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Fig 1: Photos of Bulbils from “Dougou-won” variety



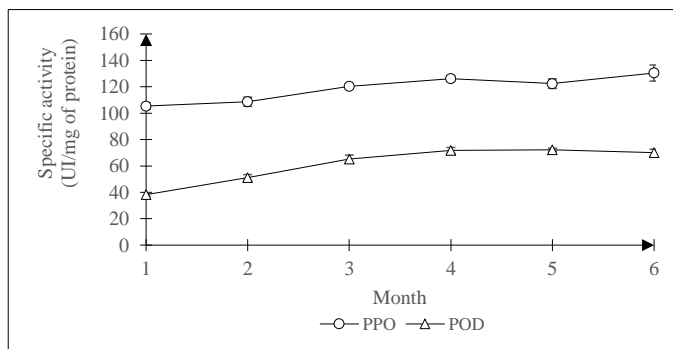


Fig 3: PPO and POD activities in bulbils from “Dougou-won” variety during the growth.

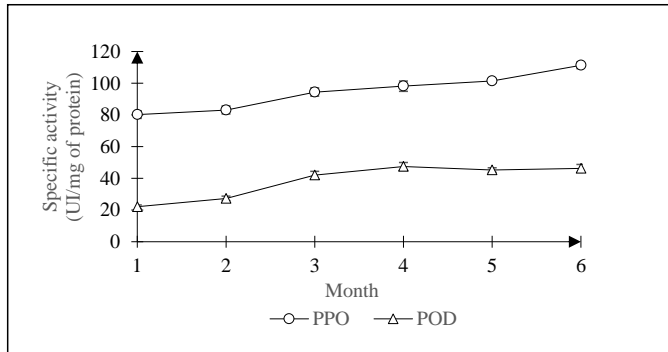


Fig 4: PPO and POD activities in bulbils from "Won-kpia" variety during the growth.

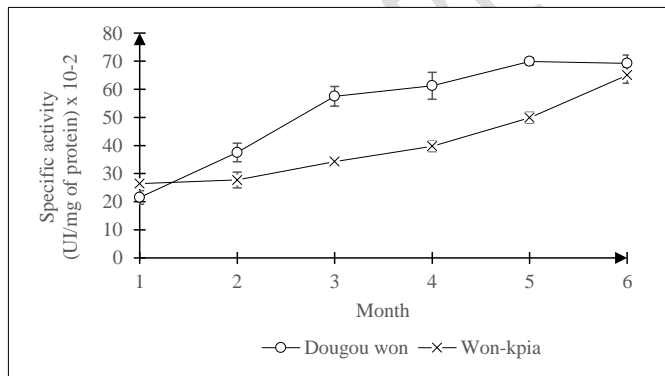


Fig 5: Acid Phosphatase activities in bulbils from "Dougou-won" and "Won-kpia" varieties during the growth.

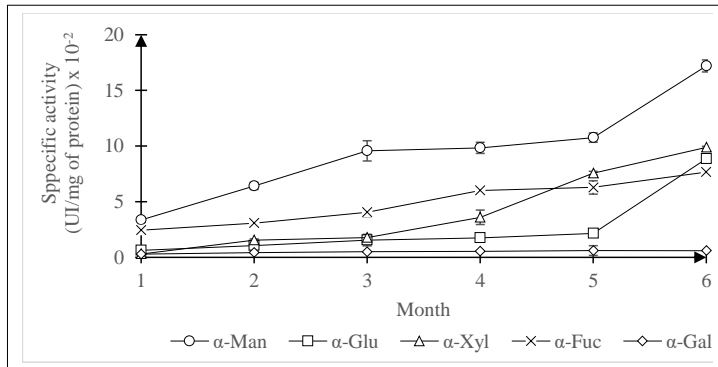


Fig 6:  $\alpha$ -mannosidase,  $\alpha$ -glucosidase,  $\alpha$ -xylosidase,  $\alpha$ -fucosidase and  $\alpha$ -galactosidase activities in bulbils from Dougou-won variety during the growth.

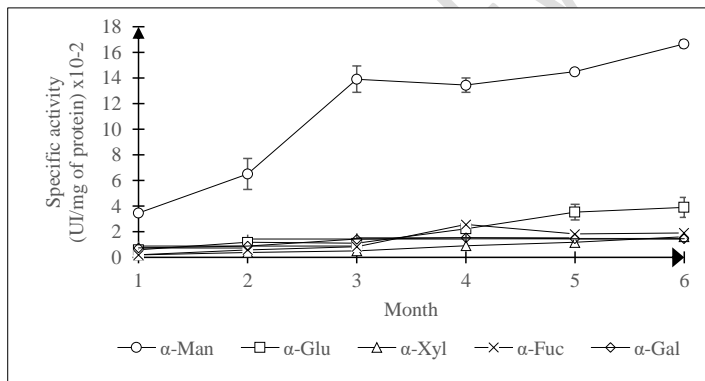


Fig 7:  $\alpha$ -glucosidase,  $\alpha$ -xylosidase,  $\alpha$ -fucosidase,  $\alpha$ -galactosidase, and  $\alpha$ -arabinosidase activities in bulbils from Won-kpia variety during the growth.

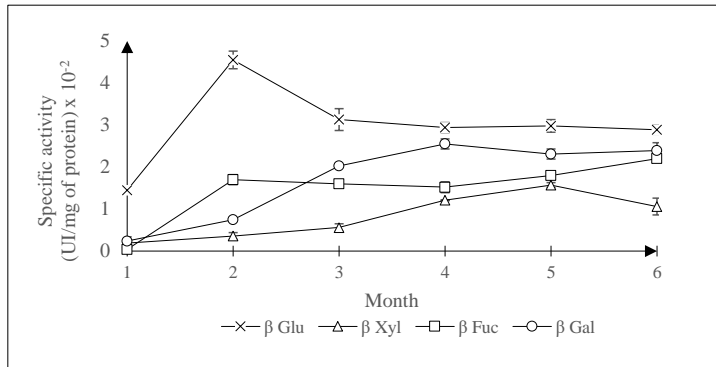


Fig 8:  $\beta$ -glucoosidase,  $\beta$ -xylosidase,  $\beta$ -fucosidase and  $\beta$ -galactosidase activities in bulbils from “Dougou-won” variety during the growth.

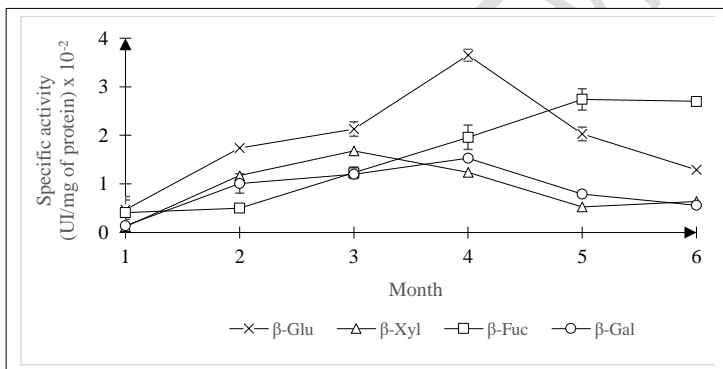


Fig 9:  $\beta$ -glucoosidase,  $\beta$ -xylosidase,  $\beta$ -fucosidase and  $\beta$ -galactosidase activities in bulbils from “Won-kpia” variety during the growth.

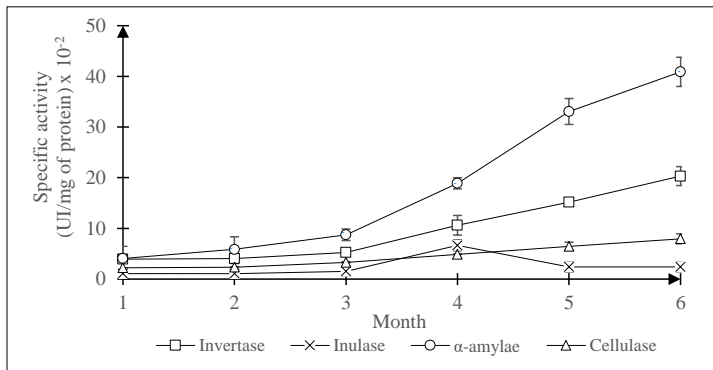


Fig 10: Invertase, inulase,  $\alpha$ -amylase and cellulase activities in bulbils from “Dougou-won” variety during the growth.

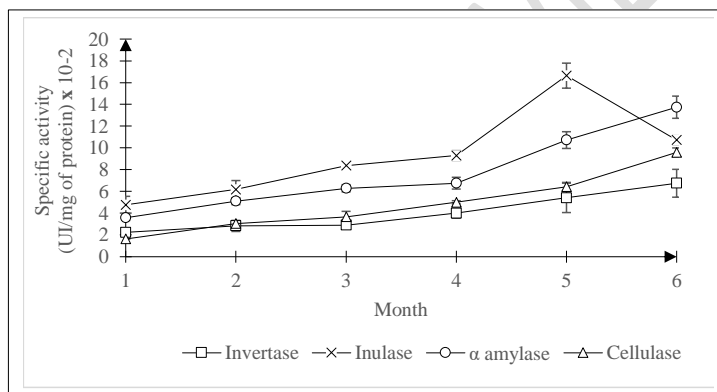


Fig 11: Invertase, inulase,  $\alpha$ -amylase and cellulase activities in bulbils from “Won-kpia” variety during the growth.