CONTRIBUTION TO THE RHIZOGENIC STUDY OF A PERENNIAL POACEE (*LYGEUM SPARTUM* L.) IN WESTERN ALGERIA

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Abstract

Through this preliminary study, we could follow up the evolution of root fragments of *Lygeum spartum* L. taken in western region (Algeria). The formation of hair root is the witness of rhizogenesis phenomenon which represents rooting phase. In our experience, the rate of rhizogenesis is different in the two regions and the size of rootlets varies from one station to another.

The rate of rhizogenesis is appreciable in nutrient agar whose percentage is 60%. The growth of rootlets is linear in northern regions (Beni-Saf, Zenata). In southern regions, the development is characterized by a more accelerated growth during the second week. The rate of rhizogenesis in the Salmonella schigella environment is about 45% in the stations of Beni-Saf, Zenata and Aouedj, the size evolution of rootlets is always linear but more late.

Keywords: *Lygeum spartum* L., Rhizome, Perennial poacee, Organogenesis, Western region (Algeria)

Introduction

Vegetative propagation is a mode that allows the propagation of genetically identical individuals (Robert and *al*., 1998), this does not involve meiosis, but another very strict process of cell division, without reworking the number of chromosomes: the Mitosis (Maarouf, 2000). Vegetative propagation occurs naturally and Artificially (Camble and Reec, 2004).

Plants biotechnologies rely mainly on in-vitro cultures. The first results interesting the Nplant tissue culture were obtained after the discovery of auxins by Went in 1934. Today, many species are concerned with the use

of in-vitro cultures, the level of the elaboration of new varieties and at the level of plants production as well, and hundreds of millions of in-vitro plants are naturalized annually all over the world in most countries even in developing countries. More than 300 of plant species which are multiplied industrially in-vitro.

The in vitro micropropagation provides considerable improvement over traditional methods with a multiplication ratio of 100 to 1000 times higher (Ochatte, 2005), this technic allows the vegetative propagation of several plants food, medicinal, horticultural (Bretaudeau, 2006).

Researches do no more use crossing technics of hybridization and cross-pollination, but aim with the help of cell cultures, protoplasts, tissues, plant organs, and thanks to genetic recombination technics as well, at protecting rapidly interesting cultivars and at creating new ones (Murashige, 1974), they are based on molecular and cellular mecanisms which are at the origin of the biological diversity (Collins, 1982 ; Kosuge *and al.*, 1983 ; Collins, 1984).

In vitro micropropagation is more or less used in the production of plants conform to the first generations of multiplication (Belguendouz, 2012). According to Le (2001), to produce genetically modified plants, regeneration plants must provide consistent

Genarally, nutrition of the root system of poacee, intersted some researchers. The biological basis of the method is the development of buds existing on plants fragments put on culture, or the induction of new buds said to be “adventitious” on explants. Thanks to its root system very developed, the *Lygeum spartum* secures the fixation and the protection of soil (Zeriahene, 1987). Actually, because of their difficulty to regenerate, the steppes sparta regress rapidly and this rapid decrease of vegetation cover causes an acceleration of desertification. The rhizogenesis is the most commonly involved organogenesis phenomenon in plant multiplication. Multiplication is the most important aspect of growth, it includes all quantitative irreversible changes which allowing the construction of plant. This is the increase in the size of roots and thus the cell size.

Very few works have been effectuated in this field, particularly on roots growth in controlled places.

Will roots make a rooting in synthetic culture places in a limited period of time? To try to answer this question we had to follow a physiological process, it is the rooting from in vitro culture of root explants of *Lygeum spartum* for this 04 steps were necessary:

- The initiation or implementation of cultures (the most delicate and difficult),
- Rooting
- The serinage or acclimatization

- Multiplication.

Methodology

- **Plant material and sampling**

The used plant species is the *Lygeum spartum* L. called in Arabic 'senagh' (Killian, 1948; Ozenda, 1958), in Spanish 'espartobasto ou albardin' (Mariano, 1876 in Chadli, 1990). It is in dense clumps and very heterogeneous (Aidoud, 1983). Its roots are in fascicle type presenting no particular orientation in their development. This one remains at lateral extension. The underground part is rhizome in internodes adventitious roots, it is very creepy and is sinks into the ground. It looks like a comb for its rectilinear growth. According to Walter (1973) the rhizome of sparta advances in 1 cm/year.

The cuttings were planted on MS places (Murashige et Skoog, 1962) supplemented in nutrient agar and agar SS (Salmonella Schigella) (**tables 1 and 2**). The used roots are taken from microscopic cuttings of *Lygeum spartum* on clumps growing in natural conditions, during spring period. A number of ten per station, root samples were inoculated, on the other hand, in sterile conditions; they are soaked in bleach for disinfection. The root fragments were cut by a sterilized blade after a rapid passage of 30 seconds in an alcohol bath at 95 ° C, they were then rinsed three times with sterile distilled water.

Roots fragments are directly dried in sterilized filter paper, then transplanted in the culture place using a sterilized clamp.

Closing boxes is essential for the prevention. The boxes steeped in culture are placed in a growth chamber with a temperature of 20-25 ° C. Boxes receive an average illumination rate of 14 hours per day because the light is crucial for triggering phenomena of organogenesis (rooting included). An extra illumination promotes rooting of some species (Margara, 1982).

Measurements are performed on root fragments each week.

Photo 1: Clump of *Lygeum spartum* L.

Table 1. Nutrient Agar

Products	Quantity
Meat extract	01 g
Yeast extract	02g
Peptone	05g
Sodium Chloride	05g
Agar agar	15g
distilled water	100ml
p H = 7.4	

Table 2. Agrar SS (Salmonella Schigella)

Products	Quantity
Meat extract	05 g
Lactose	05g
Sodium citrate	10g
Iron Citrate III	10g
bile salts	1g
Sodium thiosulfate	8.5g
Agar agar	8.5g
distilled water	12g
p H = 7.3	100ml

- **Geographical location of stations**

Rooting of vitro plants is a difficult step to realize in micro propagation. For this we have analyzed rooting capacity (elongation and development) of explants of *Lygeum spartum* from four different origins (North and South Tlemcen). The region is situated between 32°45'et 35°49' latitude north and in the West 0°32' et 1°80' longitude. The North is under coastline climatic influence at semi-arid bioclimatic stage with warm winter (the minimum temperature is at 9.5° C). The south with more continental trend is in the arid bioclimatic stage with cool winter.

Results:

Table 3: Measurements of the size of roots cultured in vitro in nutrient agar in mm

Stations	North		South	
	Beni-Saf	Zenata	Sebdou	El Aouedj
1st week	3	2,5	3	4
2nd week	4	3	5	6
3rd week	5	4	8	9
Gap-type	1,33	1,05	1,89	2,19

Figure 2: Measurements of the size of roots cultured in vitro in nutrient agar in mm

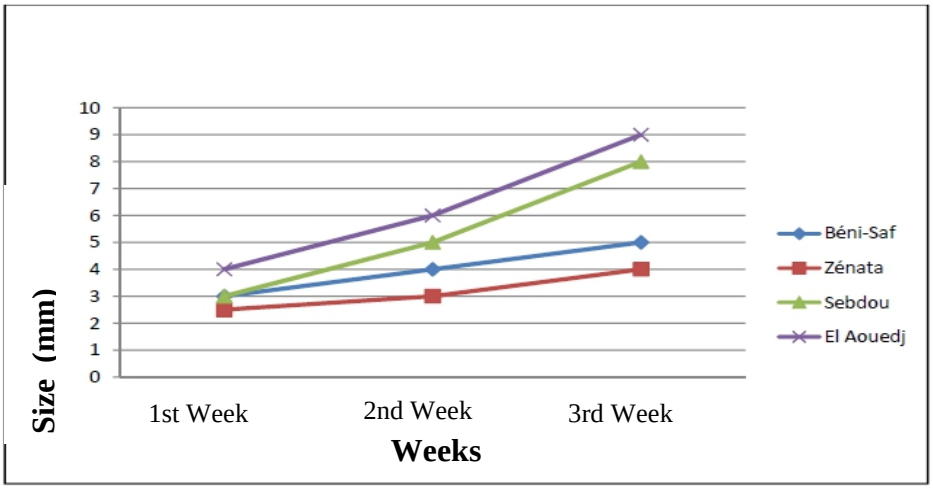
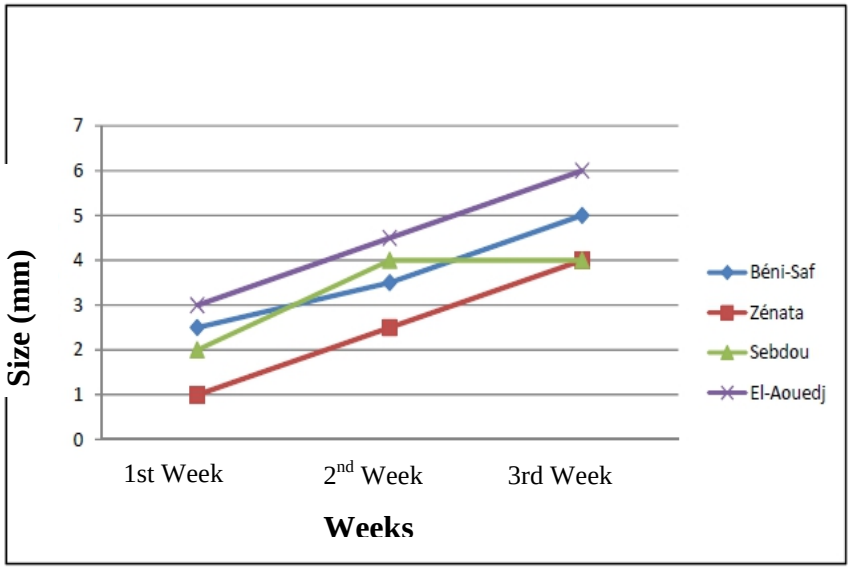
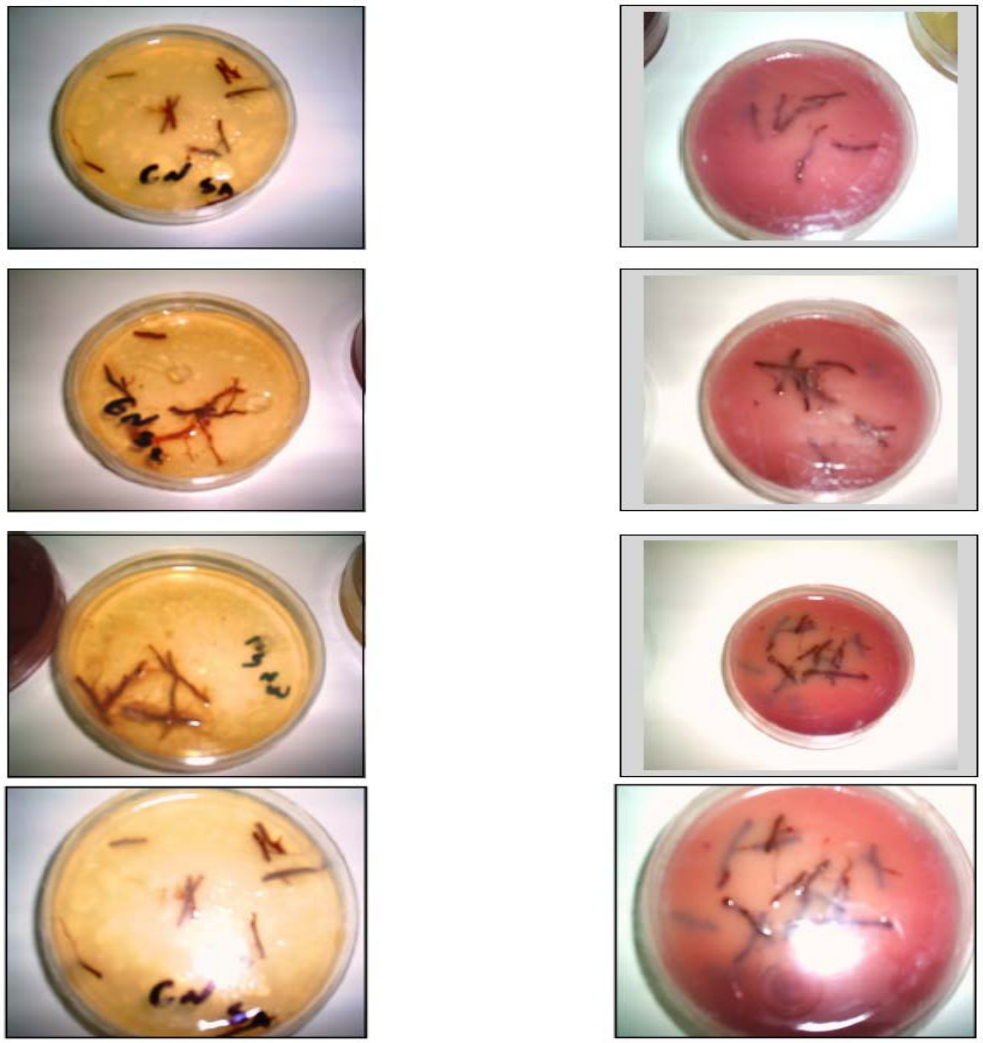


Table 4: Measurements of roots size of cultured in vitro in agar SS (*Salmonella Schigella*) in mm

Stations	North		South	
	Beni-Saf	Zenata	Sebdou	El Aouedj
1st week	2,5	1	2	3
2nd week	3,5	2,5	4	4,5
3rd week	5	4	4	6
Gap-type	1,25	4,62	4,58	1,53

Figure 3: Measurements of the size of roots cultured in vitro in agar SS (*Salmonella Schigella*) in mm





Environment : Nutrient agar **Environment: agar** *Salmonella Schigella*

Figure 4: Root growth in synthetic culture environment

Measurments were done since the 1 st week of culturing. After two to three weeks of culture, roots developed to the base of initial micros cuttings, which were taken from the seas feet, the number and length of roots developed too with a size ranging from 2 to 10 mm.

The formation of a root hair is the witness of rhizogenesis phenomenon that represents rooting phase.

In our experience, the rate of rhizogenesis is different in the two environments and the size of rootlets varies from one station to another (**Figures 2, 3 and 4**). Nutrient agar seems very supportive of this root growth.

The rate of rooting is significant, its percentage is about 60%, growth of rootlets is linear for stations in the northern zone (Béni-Saf, Zenâta). For southern zone development shows a more pronounced growth during the second week.

The rate of rhizogenesis is about 45% for Béni-Saf, Zenâta and El Aouedj zones, the change of rootlets size is always linear but expressed late. For Sebdou zone, the growth stopped the second week.

In both culture environments, rootlets of El-Aouedj region with arid bioclimate and cool winter (Bouazza, 1995 ; Benabadji *and al.*, 2009), recorded the best growth in three successive weeks. This comfortable situation for rootlets is probably due to the very favorable conditions of soil environment (presence of gypsum, aerated soil with large levels of more than 55% sand). Indeed populations of *Lygeum spartum* are denser in this station compared to other stations in the North (more than 40% as average recovery rate of vegetation). We are in optimal conditions of plant growth.

Culture environment which gave a good result was nutrient agar presumably due to favorable trophic products in quantity and quality. Rhizogenesis is not often easy to realize.

The culture environments were favorable to the development of bacteria and fungi. There is always a percentage of about 5% of infection caused by these infections.

The same seems to be insignificant contamination due to incomplete sterilization of explants probably caused by a deep infection by bacteria or fungi because sterilization of plant material is not completely realized.

The success rate is close to 60%, it confirms, however the effectiveness of the sterility of plant material.

Conclusion

Variability rooting rates between the two culture environments can be explained by the nature of these two environments which play a decisive role in vitro culture with their trophic elements.

- Rooting rate in SS environment (*Salmonella Schigella*) is less important, this means that the environment does not provide the necessary nutrients for this perennial poacée.

- For the agar nutrient, root development is more intense and stronger, although the environment provides the necessary nutrients it is not supplemented with hormones (auxins and cytokinins) cells react to hormonal content of their tissues to develop.

To conclude, we can say that these results demonstrate the ability of this pioneer species (*Lygeum spartum*), the role of the culture environment as well as the physiological state of the explant in the root organogenesis.

This work should be continued in our opinion, in the presence of other synthetic environments possibly with the addition of hormonal substances. On the other hand the collection from other more southerly study sites provide little additional information of root somatic organogenesis of the species.

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