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Culture of *Plantago* species as bioactive components resources: a 20-year review and recent applications

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Abstract.- Most of species from the worldwide distributed *Plantago* genus are greatly used as herbal medicines. Phytochemical investigations of various Plantago organs (leaves, stems) reveal their high potential to produce a wide array of bioactive secondary metabolites. So, *in vitro* cultures of *Plantago* species are managed in order to deliberately restrict the ecological factors and then to control the culture conditions. Experimental culture parameters of *Plantago* species, *i.e.* seed germination, temperature, relative humidity, light, substrates and additional nutritive solutions as well as the *in vitro* culture media and growth regulators are reviewed. The expensive optimizations of *in vitro* culture and biotransformation processes are reported. Changes in the concentration and the pattern of bioactive polyphenol compounds synthesized from *in vitro* cultures are discussed in the examined species of the genus *Plantago* in order to conclude about their potential interest as future drug candidates.

Key words : plantain - Plantaginaceae - *in vitro* culture - polyphenol - biotransformation - medicine.

Résumé.- La plupart des espèces du genre cosmopolite *Plantago* sont largement utilisées en médecine traditionnelle. Des études phytochimiques sur les différents organes des plantains (feuilles, tiges) révèlent leur fort potentiel à produire un large spectre de molécules biologiquement actives. Les techniques de cultures *in vitro* des espèces du genre *Plantago* se sont alors développées afin de restreindre les facteurs écologiques et de maîtriser les conditions culturales. Les paramètres expérimentaux de culture tels que la germination des graines, la température, l'humidité relative, la lumière, la nature des substrats et des solutions nutritives ainsi que la composition chimique des milieux de culture *in vitro* et les régulateurs de croissance additionnels ont été passés en revue. Les coûteuses méthodes d'optimisation des cultures *in vitro* et les procédés de biotransformation ont été rapportés. Les variations qualitatives et quantitatives des biomolécules de nature polyphénolique synthétisées par cultures *in vitro* de *Plantago* ont été argumentées, espèce par espèce, afin de définir l'intérêt thérapeutique potentiel de chacune.

Mots clés : plantain - Plantaginaceae - culture *in vitro* - polyphenol - biotransformation - médecine.

ABBREVIATIONS

BAP: benzylaminopurine
CA: cinnamic acid
CH: casein hydrolysate
CW: coconut water
2,4-D: 2,4-dichlorophenoxyacetic acid
DW: dry weight
E: Einstein
GA3: gibberellic acid
GB: glass beads
h: hours
IAA: (3-indolyl)acetic acid
IBA: (3-indolyl)butyric acid
Kin: kinetin
LM: light measurement
mod: modified
MS medium: Murashige and Skoog medium
NAA: α-naphtalenacetic acid
NT medium: Nagata and Takebe medium
Nutr. Sol.: nutritive solution
P: photoperiod
Pl: plantamoside
PCG: (*E*)-*p*-coumaroyl-1-O-*β*-D-glucopyranoside
RH: relative humidity
T: temperature
TDZ: thidiazuron
V: verbascoside

I. INTRODUCTION

The *Plantago* plant constitutes the principal genus of the Plantaginaceae family. About 260 *Plantago* species have been found in temperate regions and in tropical zones (Van der Aart & Vulto, 1992), including the varied ecological systems required by the plant to adapt both phenotypically and physiologically (Kuiper, 1992; Van Delden *et al.*, 1992). Many researches in diverse areas have been carried out on *Plantago* species.

Phytochemical investigations of *Plantago* species revealed the presence of various chemical constituents, *i.e.* alkaloids (Peyroux *et al.*, 1972), caffeic acid derivatives (Andary *et al.*, 1988; Murai *et al.*, 1995), coumarins (Haznagy, 1970), fats and oils (Tosun, 1995), flavonoids (Haznagy *et al.*, 1976; Galvez *et al.*, 2003), iridoids (Handjieva & Saadi, 1991; Long *et al.*, 1995; Taskova *et al.*, 2002), mucilage (Bräutigam & Franz, 1985; Kanbi & Chakraborty, 1990), polysaccharides (Samuelsen *et al.*, 1995), sterols (Afifi *et al.*, 2001), volatile substances (Kameoka *et al.*, 1979; Fons *et al.*, 1998b). Systematic significance of some bioactive components was also discussed (Taskova *et al.*, 2002; Grubesic & Vladimir-Knjezevic, 2004).

A wide range of biological activities has then been found from plant extracts or isolated compounds including wound healing activity as well as antimicrobial, antiinflammatory, antiasthmatic, antitussive, immunomodulating and antileukemic activities (De Castagno, 1970; Debrauwer *et al.*, 1989; Ravn *et al.*, 1990; Rombi, 1992; Tosun, 1995; Chang, 1997; Marchesan *et al.*, 1998; Michaelsen *et al.*, 2000; Samuelsen, 2000; Westerhof *et al.*, 2001; Chiang *et al.*, 2003a,b; Galvez *et al.*, 2003; Krasnov *et al.*, 2003; Park & Chang, 2004; Velasco-Lezama *et al.*, 2006). Bioactive component identification substantiates the effectiveness of herba plantaginis (*P. asiatica* L., *P. lanceolata* L., *P. major* L., *P. media* L.) in folk medicine. *P. ovata*, traditionally used in Egypt and India for the treatment of diarrhoea (Jasrai *et al.*, 1993) or constipation, is of significant economic interest for the Indian export trade in terms of sales of seeds (Pendse *et al.*, 1976) and mucilaginous husk (Gohel & Patel, 1997; Jain *et al.*, 1997; Babbar & Jain, 1998; Sahay, 1999).

Moreover, allergenic *P. lanceolata* pollen is of particular interest to clinicians and immuno-allergologists (Morgan *et al.*, 1995; Garcia-Ortiz *et al.*, 1996; Calabozo *et al.*, 2003). This European common and invasive species is considered by farmers as a detrimental plant (Montégut, 1983); hence the need to develop specific herbicides to protect

crops (Gange *et al.*, 1992). So, it should be noted that *Plantago* species may be considered either as intrusive herbs in agronomy or as beneficial resources for human health.

Changes in the concentrations of bioactive compounds in plantain species occurred under various natural climatic conditions. The development of processing methods to minimize the loss of biologically active constituents is then imperative. The improvement of certain species is largely due to a greater understanding of the morphological, physiological and genomic particularities of the individual species (Sen Raychaudhuri & Pramanik, 1997). The study of *Plantago* in its natural environment must be related to the concomitant study of the surrounding ecology (Clauss & Venable, 2000; Lacey & Herr, 2000; Sanderson & Elwinger, 2000a,b), allelopathy (Newman & Rovira, 1975), plant and insect-host relations (Bowers & Stamp, 1993), plant-microorganisms interactions as with bacteria, viruses and mycorrhizal fungi (Baas & Kuiper, 1989; De Nooij & Mook, 1992; Verhagen *et al.*, 1995; Staddon *et al.*, 1998; Klironomos & Moutoglis, 1999; Byrne & Mitchell, 2004; Roesti *et al.*, 2005; Blaszkowski *et al.*, 2006), pollutants (Siegel & Siegel, 1975), ozone (Reiling & Davison, 1992; Whitfield *et al.*, 1997; Zheng *et al.*, 2000; Tonneijck *et al.*, 2004), nutrition (Blacquière *et al.*, 1988; Den Hertog *et al.*, 1996), saline stress (Ferron *et al.*, 1977; Jefferies *et al.*, 1979; Königshofer, 1983; Flanagan & Jefferies, 1989; Harvey, 1989; Koyro, 2006).

In some cases such as in the study of the plant physiology and the improvement of the species, the cultivation of whole plants was carried out in the field, in greenhouses or growth chambers depending on the experimental requirements (Sanderson & Elwinger, 2000a,b; Tamura & Nishibe, 2002; Reynolds *et al.*, 2005). On the other hand, *in vitro* cultures of *Plantago* species as for *P. asiatica, P. lanceolata, P. major* and *P. ovata* have also been investigated i) to improve the *Plantago* micropropagation techniques (Barna & Wakhlu; 1988; Mathur *et al.*, 1991; Jasrai *et al.*, 1993; Tu, 1996), ii) to ameliorate the knowledge of the phenolic profile (Budzianowska *et al.*, 2004), iii) to study biotransformation processes correlated with the polyphenolic metabolism (Fons *et al.*, 1998a,c, 1999), iv) to select mutants with high stress or salt resistance, or with high bioactive compound contents (Li & Li, 2005a,b).

In this 20-year review, we outline the experimental culture conditions of *Plantago* species, *i.e.* seed germination, temperature, relative humidity, light, substrates and additional nutritive solutions as well as the *in vitro* culture media and growth regulators. The various processes involved such as callogenesis, cell culture, shoot tip regeneration and *in vitro* plant culture have been compared. Genetic improvement of species, technical maintenance of tissue culture and polyphenol accumulation of *Plantago in vitro* cultures have been examined. Variations in bioactive polyphenol pattern of *Plantago in vitro* cultures have been discussed for several species in order to conclude about the potential interest of these secondary metabolites as future drug candidates.

II. EXPERIMENTAL CULTURE PARAMETERS

The parameters influencing germination of *Plantago* seeds and seedlings growth, *i.e.* seeds age and pre-treatment, temperature, relative humidity, light, nature of substrates and nutritive solutions as well as culture media and growth regulators are discussed below.

A. *Plantago* seed origin

Plantago asiatica L., *P. lanceolata* L., *P. major* L., *P. maritima* L., *P. media* L. and *P. ovata* Forssk. are the most widely reported species in the literature. The seeds of *Plantago* used for most of the research programmes usually were provided from private collections (Staal *et al.*, 1991) and Botanical Garden or Research Institute collections (Makowczynska & Andrzejewska-Golec, 2000; Budzianowska *et al.*, 2004); they were also harvested locally from wild plants (Flanagan & Jefferies, 1989; Harvey, 1989; Rouhier & Read, 1998; McCloud & Berenbaum, 2000; Wolff *et al.*, 2000) or purchased from industrial producers (Staddon *et al.*, 1998, 1999; Hodge *et al.*, 2000).

B. Age of seeds and impact of this trait

At the time of planting, the age of seeds was not specified most of the time (Harvey, 1989; Maathuis & Prins, 1990; Rozema *et al.*, 1992; Forbes *et al.*, 1996). Several authors indicated that the seeds had been harvested during the same year (Jefferies *et al.*, 1979; Pons, 1991b; Horner & Bell, 1995; Saker & Kawashity, 1998) or in the previous two or three years (Gagnaire *et al.*, 1975; Flanagan & Jefferies, 1989).

In 1992, Blom reported the results of a study carried out on the influence of the seed age (from 0 to 6 years) on the germination of five *Plantago* species (*P. coronopus* L., *P. lanceolata*, *P. major, P. maritima* and *P. media*). For *P. maritima*, the age of the seeds did not change the capacity to germinate. For the other four species, the germination rate of the seeds harvested later was significantly lower than those of the seeds harvested earlier, *i.e.* at one or two-year old. Contrary to the seeds of *P. lanceolata*, the germination of the other species decreased after four or five years. This research demonstrates that the use of one-to-four year old seeds from the genus *Plantago* increases the capacity to germinate. While studying storage conditions of different populations of *P. albicans* L. (Morocco and Tunis), Puech *et al.* (1998) observed that seeds of these populations kept in the dark over a three-year period retained the same capacity to germinate.

C. Pre-germination treatments of the seeds

Most often, seeds were used straight for germination. Infrequently, the seeds were treated with gibberellic acid (10^{-4} M) in order to help break the dormancy (Horner & Bell, 1995). It was also found that KNO_3 was effective in stimulating *P. major* in non-optimal light conditions (10^{-2} M) (Pons, 1992). McNeil and Duran (1992) also studied the effect of KNO_3, gibberellin and temperature on the germination of *P. ovata.* Gibberellin as well as KNO_3 or thiourea are known to increase the germination of various vegetable species (Boutherin & Bron, 1989).

Moreover, Sagar and Harper (1960) and later Blom (1992) found the usefulness of pre-chilling *P. major* seeds at 5 °C for seven to fourteen days. This pre-treatment notably increased the germination of this species, contrary to *P. lanceolata* or *P. media.* Pons (1992) found that cold stratification of *P. lanceolata* and *P. major* seeds favoured the germination of both species. In spite of promising results, these two pre-treatments were mainly used for dormancy and germination studies of *Plantago* seeds. Low-temperature pre-treatment is employed not only to promote seed germination but also to increase root and shoot induction on callus cultures or to overcome, bulbs and even woody plant dormancy (George, 1993). More-or-less prolonged cold stratification or pre-chilling treatments (from few weeks to several months according to the species) in addition to scarification or dessication are frequently reported to enhance seed germination (Boutherin & Bron, 1989).

For mycorrhization studies or *in vitro* cultures, seeds were surface sterilized in mercuric chloride (0.05%-0.1%) for 2-10 min (Wakhlu & Barna, 1989; Jasrai *et al.*, 1993;

Pramanik *et al.*, 1996), in sodium hypochlorite (from 3.6 to 5.25%) for 10-30 min (Fons, 1998; Fons *et al.*, 1998a,c; Saker & Kawashity, 1998) or in calcium hypochlorite (Bräutigam & Franz, 1985), sometimes in combination with 70% ethanol from 30 sec to 2 min (Budzianowska *et al.*, 2004). These disinfectants are used more often than not for *in vitro* cultures (Zrÿd, 1988; Boccon-Gibod, 1989b; George, 1993).

D. Growth temperature and its influence

In most cases, sowing or seedling growth temperatures ranged from 15 °C to 25 °C (Tables I-II) with occasional daily fluctuations. Higher temperatures, *i.e.* 27 °C or 25 °C (Miao & Bazzaz, 1990) and 36 °C (Gagnaire *et al.*, 1975) were also reported, respectively. Different authors (Sagar & Harper, 1960; Blom, 1992) have demonstrated that the percentage of germination for *P. media* and *P. major* seeds dramatically increased from 15 °C to the optimal temperature of 25 °C whereas the germination of *P. coronopus, P. lanceolata* and *P. maritima* was temperature-independent. McNeil and Duran (1992) studied the influence of both temperature and gibberellic acid on the germination, development and flowering of *P. ovata.*

Temperatures from 15 °C to 25 °C used for *Plantago* studies are frequent in culture chambers or in glasshouses (Table III) and are species-dependent (Favreau, 1980; Boutherin & Bron, 1989; Boccon-Gibod, 1989a). But in order to enhance the plant growth, *in vitro* culture temperatures are often higher than the temperatures occurring naturally in the field (George, 1993). For *in vitro* cultures managed in culture chambers, the temperatures mentioned for plants, organs or tissues cultures are frequently the same but for certain plant species optimal temperatures have been determined as culture type-dependent (George, 1993).

E. Relative humidity

The effects of 8 to 21% moisture on seed germination were investigated by Blom (1992). Only *P. coronopus* and *P. lanceolata* showed a high percentage of germination (from 70 to 80%) at the lowest moisture content. For all other species, germination increased with moisture content and optimum results were reached at 21% moisture.

For glasshouse cultures or *in vitro* cultures of the different *Plantago* species, the relative humidity varied from 40 to 100% (Tables I-III). Experimental glasshouses should have a high relative humidity in order to maximise seed germination and rooting (Favreau 1980; Boutherin & Bron, 1989). The theoretical relative humidity in sealed culture vessels is 98-100% for *in vitro* cultures (Boccon-Gibod, 1989a). However, if the vessel is simply covered with cotton wool or filter paper, exchanges can take place with the growth room thus dramatically reducing the relative humidity within the culture vessel (50%). The minimal relative humidity that provides suitable growing conditions for organs and plants is 70%, moisture level generally produced in growth chambers (George, 1993).

F. Light as culture growth factors

In the same way as temperature and humidity, light is a major factor for culture growth conditions (Kozai *et al.*, 1997). There are, in fact, three parameters associated with light as the photoperiod, the wavelength and the light intensity.

Germination of most of *Plantago* seeds was carried out under daylight conditions but certain authors preferred the use of dark conditions (Siegel & Siegel, 1975; Wakhlu & Barna, 1989). Blom (1992) reported that both *P. lanceolata* and *P. media* had the ability to germinate in the dark, *i.e.* 76-100% and 46-73%, respectively. Conversely, *P. major* either did not germinate in the dark (Blom, 1992) or only germinated to a slight extent, *i.e.* 5%

(Sagar & Harper, 1960). Light did not favour the germination of *P. lanceolata* but increased the germination of *P. coronopus, P. major* and *P. media* (Blom, 1992). These results were noticeably different from these of Pons (1991a) demonstrating that germination of *P. lanceolata* and *P. media* in darkness did not exceed 50% and 25%, respectively whereas in daylight these percentages reached 100% for both species. That could be explained by the use of varying seed populations. Intra-specific variations between different populations could be explained by the adaptation of plants to various ecosystems.

Hence, the impact of environment on the capacity to germinate of *P. albicans* has been highlighted by Puech *et al.* (1998). Optimal germination of seeds provided from various ecological environments (Tunisia, Morocco) was obtained at 25 °C (versus 20 °C) in dark (versus light). The germination capacity of hexaploid populations from humid environments (Siliana, Maktar) was elevated in dark conditions (> 70% at 20 °C and 25 °C) and practically none under daylight conditions (at 20 °C or 25 °C). However, the highest percentage of germination within tetraploid populations in arid environments (Chebkanna, Medenine, Gafsa) was much lower than for hexaploid populations: < 60% at 25 °C in the dark, < 51% at 25 °C in daylight, < 35% at 20 °C in the dark and < 21% at 20 °C in daylight.

Photoperiod variations

The growth and development of both *Plantago* seedlings and plants in growth chambers or in glasshouses were mainly carried out over a 12-to-16 hour photoperiod (Tables I-III) and sometimes over an 18-hour photoperiod (Newman & Rovira, 1975). Lacey and Herr (2000) reported an 8-hour photoperiod for two months to induce vegetative growth of *P. lanceolata* and then a 16-hour photoperiod to promote flowering. Boccon-Gibod (1989a) showed that, for *in vitro* culture of plants belonging to many genera, photoperiods of 16-18 hours were commonly used.

George (1993) pointed out that extended exposure to daylight had the same kind of effect on plant tissue cultures as exposure to red light. Extended daylight exposure (for example during long summer days) leads to a higher auxin content in the plant than does reduced daylight exposure (short days). The photoperiod and irradiation influence both the flowering of the plant and the growth of vegetative organs. For the same plant species, different specific photoperiods can be used to promote shooting, rooting or flowering. (George, 1993). In glasshouses, the supply of light generally depends on the season and more specifically on the total maintenance cost (Favreau, 1980).

Wavelength range

According to Pons (1992), the germination rate of *P. lanceolata* and *P. major* increased as the ratio of red-to-far red increased in the light source, and was temperature-dependent, *i.e.* temperature of seed stratification or storage and temperature of the test requirement. Moreover, this ratio induced morphological and growth variations of *P. lanceolata* (Van Hisberg, 1997; Van Hinsberg & Van Tienderen, 1997). Red light (2 $W.m^{-2}$ for 2 hours daily) was also used to initiate the flowering of *P. lanceolata* (Freijsen & Otten, 1993).

Many studies carried out at various wavelengths (UV, blue light, green-yellow light, red light) with different tissues from *Plantago* species, showed that callogenesis, caulogenesis or rhizogenesis could be specifically induced or inhibited by a given waveband (George, 1993). However, in most of experiments with *Plantago* cultures, wavelength was not modified for the different stages of tissue cultures (Tables I-III); the wavelength was not, indeed the subject of these experiments. Growth chambers are usually equipped with fluorescent

tubes as cool white or Grolux tubes (George, 1993) but this detail is not generally mentioned in the publications.

Measurement of light energy

Varied units are adopted by the authors for the expression of light quantification (Tables I-II) that make difficult the comparison of results reported in literature (Desvignes, 1991). In fact, three units are commonly cited, *i.e.* illuminance (lux), irradiance ($W.m^{-2}$) and photon flux density ($\mu E.m^{-2}.s^{-1}$, $\mu mol.m^{-2}.s^{-1}$). George (1993) provided factors for converting between these light units: 1 $\mu E.m^{-2}.s^{-1} = 0.215$ $W.m^{-2}$. It is also accepted that 1000 lux = 5 to 10 $W.m^{-2}$ (Demeyer *et al.*, 1982; Boccon-Gibod, 1989a) for the daylight but this converting factor is not constant because the energy of the light varies with the wavelength of the source (George, 1993). The light intensity of the daylight varies from 200 to 700 $W.m^{-2}$ (50000-150000 lux) and the flux densities of controlled environment cultures are at least ten times lower. For experimental cultures of *Plantago* species, illuminance of 1500- 16000 lux or irradiance of 15-450 $\mu mol.m^{-2}.s^{-1}$ were recorded (Tables I-II).

In greenhouses, the light conditions depend on the plant species, the seasons, the light intensity of sunshine – the minimum commonly being around 2000 lux (Reynoird & Vidalie, 1989). In controlled-environment chambers (Boccon-Gibod, 1989a), the irradiances supplied to the plants usually vary between 5 and 25 $W.m^{-2}$ (1000-5000 lux), though the optimal light conditions will be based on the individual plant species, the tissue or organ cultured (callus, root, plantlet) and the culture step. The light intensity therefore can range from total darkness to very high illuminance as 10000-15000 lux (George, 1993).

G. Solid substrates and additional nutritive solutions

As listed in Table III, *Plantago* seeds were sown on cotton (Wakhlu & Barna, 1989; Horner & Bell, 1995), filter paper (Fitter & Stickland, 1991; Staddon *et al.*, 1999), Petri dishes (Siegel & Siegel, 1975), wet glass beads (Freijsen & Otten, 1987), commercial germination medium (McCloud & Berenbaum, 2000) or directly on plant growth substrate.

Then, substrates used for *Plantago* cultures are various such as vermiculite (Smakman & Hofstra, 1982; Reekie & Bazzaz, 1992), glass beads (Van Hinsberg, 1997), sand (Rouhier & Read, 1998; Klironomos & Moutoglis, 1999; Clauss & Venable, 2000), soil (Pons, 1991a; Shem-Tov *et al.*, 1999), sterilised soil (Fonseca *et al.*, 1997), sandy soil (Schippers & Olff, 2000), composts (Sykes & Wilson, 1990; Van Damme, 1991; Silva & Teresa, 1992; Whitfield *et al.*, 1997; Zheng *et al.*, 2000), commercial soil mixture (Dijkstra & Kuiper, 1989) or substrate mixtures, *i.e.* sand/soil/sawdust, soil/Turface®/sand (Fajer *et al.*, 1991), sand/Terragreen® (Staddon *et al.*, 1998, 1999; Hodge *et al.*, 2000), soil/peat/perlite (McCloud & Berenbaum, 2000).

Indeed, both nature and structure of the substrate influence seed germination. Sagar and Harper (1960) showed major differences in the percentage of seed germination of *P. lanceolata* according to the nature of the culture substrate, *i.e.* 45% on filter pad, 54% in vermiculite and 72% in soil. The interaction between the particles of substrate and seeds was seed size-dependent (Blom, 1992). The germination of *P. coronopus, P. lanceolata, P. major, P. maritima* and *P. media* seeds on various sizes of glass beads (from 0.01 mm to 1.23 mm) was studied by Blom (1992). The germination of large seeds (*P. lanceolata, P. maritima* and *P. media*) increased with the size of the glass beads whereas the opposite was found with the smallest seed species. Seedling mortality was lower with the largest bead size.

In field conditions, soil compaction, moisture, structure of the upper soil layers as well as trampling and flooding influenced differently each species of *Plantago* (Blom, 1992). Growth chamber, greenhouse and field studies were conducted to compare the emergence of *P. lanceolata* seedlings from 1, 3 or 6 cm planting depth (Sanderson & Elwinger, 2000b): for rapid establishment, seeds of *P. lanceolata* should not be sown deeper than 1 cm. Shem-Tov *et al.* (1999) studied *P. coronopus* seed germination in the Negev Desert (Israel). They demonstrated that there was a positive correlation between the soil carbon content, the rainfall gradient and the seed germination inhibition.

The physical characteristics of substrates (porosity, particle size...) contributed to the retention of water or nutritive solution (Gras, 1987). The particle size of the substrate should be proportional to the size of the seeds (Boutherin & Bron, 1989) and, as already stated, can influence germination (Blom, 1992), plant rooting and oxygenation of the roots (Lemaire *et al.*, 1989).

Substrates can be used as simple supporting materials with the addition of nutritive solutions, or they can provide both support and nutrition to the plant (Morard, 1995). In the former case, the substrate should be chemically neutral (vermiculite, glass beads, sand, rock wool, Pozzuolana) while in the latter case (soil, compost, peat, bark), exchanges with cultures are actively sought. The most widely used substrates vary in their chemical properties depending on their nature (André, 1987). However, the substrates that provide both support and nutrition are often less stable in time and they deplete more or less rapidly.

Typical nutritive solutions reported for *Plantago* are listed in Table III as Hoagland solution (Miao *et al.*, 1991; Rozema *et al.*, 1992; Hoagland & Snijder, 1993), Johnson Solution (Johnson *et al.*, 1957; Flanagan & Jefferies, 1989), Lewis and Powers solution (Lewis & Powers, 1941; Smakman & Hofstra, 1982), Steiner solution (Steiner, 1968; Lotz *et al.*, 1990) and other nutrient solutions (Cooper & Etherington, 1974; Cooper, 1976).

H. *In vitro* culture media and growth regulators

For *in vitro* cultures, substrates consisted of culture media, liquids or solidified with agar, gelrite, Phytagel or isubgol (Gohel & Patel, 1997; Babbar & Jain, 1998). Hence, auxins, cytokinins and gibberellins were used to influence callogenesis, caulogenesis, rhizogenesis or growth of *Plantago* species in experimental cultures (Dijkstra & Kuiper, 1989; Dijkstra *et al.*, 1990; Makowczynska & Andrzejewska-Golec, 2000, 2006).

Bräutigam and Franz (1985) illustrated the utility of NT medium (Nagata & Takebe, 1971) for callus induction of embryos obtained from seeds of *P. lanceolata* and *P. psyllium* L., though MS medium (Murashige & Skoog, 1962) has mainly been reported with the addition of growth regulators for the *in vitro* culture of *Plantago* species (Table II). Normal MS medium was usually used (Pramanik *et al.*, 1996; Saker & Kawashity, 1998; Makowczynska & Andrzejewska-Golec, 2000), but MS medium modified was also mentioned for different micropropagation steps, *i.e.* callus initiation (Budzianowska *et al.*, 2004), axillary shoots proliferation (Mederos-Molina, 1994) and root induction (Mederos *et al.*, 1997/1998; Fons *et al.* 1999; Li & Li, 2005a).

Plantago asiatica in vitro *culture*

The micropropagation of Asian plantain was achieved through shoot-tip multiplication adding 0.1 $mg.dm^{-3}$ IAA and 1 $mg.dm^{-3}$ BAP (Makowczynska & Andrzejewska-Golec, 2003) or after callus induction with 2,4-D alone (Tu, 1996) or with BAP (Makowczynska & Andrzejewska-Golec, 2000; Makowczynska *et al.*, 2004). After callus culture, somatic organogenesis (shoot and root regeneration) and somatic embryogenesis occurred with

various combinations of BAP with 2,4-D and NAA (Tu, 1996; Makowczynska & Andrzejewska-Golec, 2000).

Plantago lanceolata in vitro *culture*

Various explants of *P. lanceolata* were investigated for plant regeneration and micropropagation: leaf and root (Budzianowska *et al.*, 2004) as well as hypocotyl and cotyledon (Khawar *et al.*, 2005). The first explant group allowed the multiplication of leaf rosettes on MS medium supplemented with IAA (11.42 µM) and Kin (9.29 µM) whereas the second explant group produced first shoot regeneration (MS supplemented with 3.33 µM BAP and 1.34 µM NAA) and then micropropagation (MS supplemented with 0.45 µM TDZ and 0.09 µM IBA). Micropropagated plants were rooted with MS medium with 5.71 µM IAA by the first team cited above and with MS medium + 1.16 µM Kin + 0.98 µM IBA + 2.69 µM NAA by the second team who noticed that TDZ completely inhibited root development.

Callus induction was achieved by Bräutigam & Frantz (1985), and Budzianowska *et al.* (2004) as mentioned in Table II.

Hairy root cultures (transformed by *Agrobacterium rhizogenes*) generated from root and leaf explants of *P. lanceolata* were initiated and subcultured on a modified MS medium (N/5) (Fons, 1998; Fons *et al.*, 1999). Agropine-type strains of *A. rhizogenes* were used for the transformation of explants and roots appeared 8 days later. A statistic covariance study was carried out after counting the hairy roots: from 5 to 20% of leaf fragments and from 46 to 80% of root fragments exhibited rhizogenesis. Nevertheless, the age of seedlings when cutting the fragments (from 22 to 57-day old) did not influence rhizogenesis. The rhizogenesis technique applied on root fragments showed efficiency as 66.7% (Fons, 1998).

Plantago major in vitro *culture*

Saker and Kawashity (1998) experimented with different combinations of growth regulators (2,4-D, IAA, IBA, NAA and Kin) added to MS medium in order to induce callogenesis, caulogenesis or rhizogenesis in leaf explants of *P. major.* Optimum results (Table II) for callus proliferation (2 $mg.l^{-1}$ 2,4-D + 0.5 $mg.l^{-1}$ Kin), shoot proliferation (0.5 $mg.l^{-1}$ IAA + 1 $mg.l^{-1}$ Kin) and root proliferation (2 $mg.l^{-1}$ IBA + 1 $mg.l^{-1}$ NAA) were obtained by modulation or inversion of the cytokinin/auxin balance. Li and Li (2005a) used also leaf explants to induce callus proliferation and then bud and root formation using successively NAA, BAP and modified MS (Table II).

Equally from cultured calli of *P. major*, roots and then shoot-buds were produced by subcultures on MS + 1 $mg.l^{-1}$ 2,4-D + 0.5 $mg.l^{-1}$ BAP and then on MS + 0.5 $mg.l^{-1}$ BAP (Mathur *et al.*, 1991).

Shoot proliferation and rooting of cultures of *P. major* shoot-tips were then obtained on MS modified + 0.5 µM BAP and then MS modified + 1 µM NAA (Mederos *et al.*, 1997/1998). It should be mentioned that the previous *Plantago* research team was the only one inducing rhizogenesis from callus before shoot regeneration using a low cytokinin/auxin ratio (Table II).

Plantago ovata in vitro *culture*

From shoot apices of *P. ovata*, shoot induction and then rooting were obtained from cultures on MS medium + 4.6 µM Kin + 0.05 µM NAA and then in 1/2 MS + 5 µM IBA + 0.05 µM Kin (Barna & Wakhlu, 1988). Shoot tip multiplication and then rooting of the shootlets were described by Pramanik *et al.* (1995).

According to authors, callogenesis was initiated from shoot bud, leaf segments or hypocotyls of *P. ovata* on MS medium with i) 4.5 µM 2,4-D and 2.3 µM kinetin (Das & Sen Raychaudhuri, 2001), ii) 1 µM 2,4-D and 2 µM kinetin (Jasrai *et al.*, 1993), iii) 4.5 µM 2,4-D and 4.6 µM kinetin (Wakhlu & Barna, 1988, 1989), iv) 0,4 $mg.l^{-1}$ NAA and 0.4 $mg.l^{-1}$ BAP (Pramanik *et al.*, 1994, 1996).

It may be noted that both former research teams used inverse proportions of 2,4-D and kinetin and both latter teams used equal contents of auxins and cytokinins for callogenesis induction. According to Jasrai *et al.* (1993), 1 µM kinetin allowed good callus growth; however the best results were produced with twice the amount of cytokinin. From these calli of *P. ovata*, micropropagation was achieved through three different methods.

Firstly, cell suspensions followed by embryogenesis were obtained by subcultures of these calli in the same medium (Jasrai *et al.*, 1993). Then, germination of the embryoids and plantlet formation were induced on MS medium with a higher concentration of kinetin (4.6 µM). The low conversion of the somatic embryos was attributed to an over-exposure to auxin during induction of embryos (Jasrai *et al.*, 1993). Das and Sen Raychaudhuri (2001) highlighted that casein hydrolysate (2 $g.l^{-1}$) and coconut water (10%) enhanced the growth of embryogenic cultures in combination with BAP and NAA.

Secondly, caulogenesis was initiated from the calli with high value of cytokinin/auxin ratio as i) NAA (0.05 µM) + kinetin (18.4 µM) by Wakhlu & Barna (1988, 1989), ii) IAA (0.2 $mg.l^{-1}$) + BAP (5 $mg.l^{-1}$) by Pramanik *et al.* (1994, 1996).

Finally, callus-derived roots followed by shoot induction were produced on a single MS medium + 11.4 µM IAA + 0.9 µM kinetin (Barna & Wakhlu, 1989). To avoid vitrification, the shoots were excised and transferred on 1/2 MS + IBA for rooting. This was the single case of callus-derived root obtaining followed by shoot regeneration and root induction using three different culture media.

The rooting media reported in literature were MS medium without growth regulators (Pramanik *et al.*, 1994, 1995, 1996), MS medium added with only auxin (Wakhlu & Barna, 1988, 1989; Mederos *et al.*, 1997/1998) or added with auxin and small amounts of cytokinin (Barna & Wakhlu, 1988).

III. IMPROVEMENTS AND APPLICATIONS OF *PLANTAGO IN VITRO* CULTURES

The expensive optimizations of *in vitro* cultures and biotransformation processes as well as changes in polyphenol pattern of *Plantago in vitro* cultures are reported in order to use these engeenering techniques as bioactive components suppliers.

A. Genetic improvement of species and technical maintenance of tissue cultures

Several effective micropropagation protocols have been developed. From callus cultures of *P. ovata*, Barna and Wakhlu (1988) then Wakhlu and Barna (1988) obtained good results in establishing regenerated plants in potted soil or in a sand/soil/sawdust substrate mixture. These regenerated plants were diploids and resembled to control plants ($2n = 8$). The data indicated much promise for the genetic improvement of this *Plantago* species and ultimately for its production in India (Jasrai *et al.*, 1993). Pramanik *et al.* (1995, 1996) have shown that esterase and superoxide dismutase isozymes act as biochemical markers which differentiates multiplying shootlets from control or regenerating plants.

On the other hand, Mathur *et al.* (1991) demonstrated the utility of mineral oil in reducing shoot-bud regeneration from the callus of *P. major* (180 days instead of 45-60 days for control) with a regeneration ratio of 82%. The mineral oil overlay technique could appre-

ciably reduce the cost of maintenance of tissue cultures. Moreover, the mineral oil used over alginate beads was reported to protect encapsulated explants from desiccation and microbial attack.

B. Bioactive component accumulation in *in vitro* cultures

Primary metabolites (sugars) and especially secondary metabolites (polyphenols as flavonoids and phenylethanoid glucosides) patterns of genus *Plantago in vitro* cultures are investigated using various chromatographic analyses. Then, the production of both well known and new synthesized polyphenols is reported with biotransformed root cultures of *P. lanceolata.*

Chemical analyses of primary and secondary metabolites

Regarding chemical analyses of *Plantago in vitro* cultures, Bräutigam and Franz (1985) reported significant differences in sugar content between calli, cell suspensions, leaves or seeds of *P. psyllium* and *P. lanceolata.*

Saker and Kawashity (1998) found the flavonoid content of *P. major* organ-dependent, *i.e.* calli (ranging from 0% DW to 20% DW), plantlets (30% DW), shoot cultures (10% DW), root cultures (40% DW) and *ex vitro* plants (60% DW). Higher levels of flavonoids were recorded in *ex vitro* plants and undifferentiated tissues contained less than organized tissues. Such differences in the levels of secondary metabolites between whole plants and cell or tissue cultures have often been described (Hamill *et al.*, 1987; Ishimaru *et al.*, 1990; Stepan-Sarkissian, 1991; Zafar *et al.*, 1992). In addition, *P. major* calli with the highest growth rates contained the lowest levels of flavonoids. This is why for industrial production of secondary metabolites a great number of protocols involve two steps: the first one involves tissue or cell culture in a growth medium followed by a subculture step using a production medium (Toivonen *et al.*, 1990; Stafford, 1991; Stepan-Sarkissian, 1991; Pitta-Alvarez & Giuletti, 1995).

Later, Budzianowska *et al.* (2004) analyzed phenylethanoid glucosides of leaves (from *in vitro* propagated plants and from micropropagated plants transferred to field cultivation) and of two lines of calli (root or leaf-derived calli) from *P. lanceolata.* Nine phenylethanoid glucosides were identified from this species as the well known compounds, verbascoside, plantamoside and lavandulifolioside as well as six novel compounds for this species, *i.e.* leucosceptoside A, martynoside, desrhamnosylverbascoside, desrhamnosylisoverbascoside, plantainoside D (isoplantamoside) and a conjugate of (*E*)-*p*-coumaroyl, 4-hydroxyphenethoxy and glucopyranosyl moieties (lancetoside). The latter compound was described for the first time in the nature. Verbascoside (V) and plantamoside (Pl) were found in all the plant materials but the former was mainly detected in leaves (1.78-10.43% DW) and the latter, mainly identified in calli (1.19-2.84% DW).

Biotransformation processes and production of polyphenols

Studies carried out on *P. lanceolata in vitro* cultures demonstrated that from day 30 to day 98, cumulated levels of Pl and V were four-to-ten higher in roots than in leaves (Fons, 1998). In fact, Pl (2.4 $mg.g^{-1}$ DW) and V levels (8.8 $mg.g^{-1}$ DW) were both remarkably low and stable in the leaves. In the roots, the storage of V was greater (26 $mg.g^{-1}$ DW) and the storage of Pl increased with the age of the seedlings (from 30 to 100 $mg.g^{-1}$ DW). These two compounds constituted from 4 to 14% of root dry weight with 50% to 75% of Pl. In transformed root cultures, the accumulation of Pl (30 to 80 $mg.g^{-1}$ DW) and of V (6 to 12 $mg.g^{-1}$ DW) were globally equal to these reported for the roots of whole seedlings (Fons, 1998; Fons *et al.*, 1998a,c, 1999).

Cinnamic acid (CA) has been shown to be integrated into the polyphenol pathway of plant species (Douglas *et al.*, 1992; Bate *et al.*, 1994; Herrmann, 1995). The biotransformation of CA by ribwort plantain cultures (seeds, from 30 to 98-day old seedlings, hairy root cultures) has also been investigated (Fons, 1998; Fons *et al.*, 1998a). The addition of CA to the culture medium of *P. lanceolata* did not modify the levels of Pl and V in seedlings. However, new phenolic derivatives were isolated and (*E*)-*p*-coumaroyl-1-O-*β*-D-glucopyranoside (PCG) was identified (Fons *et al.*, 1998c). Only small amounts of PCG were detected in the leaves (< 0.6 $mg.g^{-1}$ DW) whereas from 8 to 22 $mg.g^{-1}$ DW of PCG were found in the roots fed with 0.12 to 1 mM CA, respectively. However, CA showed toxic effects on the plantlets at the higher concentration.

The addition of 0.1 mM CA in 42, 50 and 58-day old root cultures of transformed cultures did not induce any toxicity and did not modify both V (7-11 $mg.g^{-1}$ DW) and Pl (40-75 $mg.g^{-1}$ DW) levels which were close to those reported in roots of whole plants (Fons, 1998; Fons *et al.*, 1998a,c, 1999). Maximal levels ranging from 2 to 6 $mg.g^{-1}$ DW of PCG were found in hairy root cultures (Fons, 1998; Fons *et al.*, 1999).

Such biotransformation of precursors of aromatic compounds was previously reported for several plant species, *i.e. Cestrum poeppigii, Coffea arabica, Nicotiana tabacum, Phaseolus vulgaris, Solanum* sp. (Molderez *et al.*, 1978; Moriguchi *et al.*, 1988; Ushiyama *et al.*, 1989; Edwards *et al.*, 1990).

IV. CONCLUSION AND PERSPECTIVES OF *PLANTAGO IN VITRO* CULTURES

Plantago is a polymorphic genus of the Plantaginaceae family constituted of more than 260 species worldwide distributed. Phytochemical investigations of *Plantago* species reveal their high potential to produce a wide array of bioactive secondary metabolites, *i.e.* iridoids, polyphenols, polysaccharides and sterols (Li *et al.*, 1995; Samuelsen *et al.*, 1995; Afifi *et al.*, 2001; Grubesic & Vladimir-Knjezevic, 2004) that have utility as supplemented feed (Tamura *et al.*, 2002) and as drugs to treat human diseases (Chiang *et al.*, 2003a,b; Hetland, 2003).

Various studies on *Plantago* species in both natural and controlled environments have been lead. In the first ones, environmental factors, *i.e.* micro-organisms, allelopathy, trampling or flooding can not be easily mastered. These ecological factors are deliberately restricted in greenhouses or growth chambers.

Regarding the culture conditions of *Plantago* species, most of the well known parameters influencing seed germination and seedlings growth belong to both physical and chemical types, *i.e.* temperature, humidity, light, substrates with or without additional nutritive solutions and culture media with or without growth regulators (Gjerstad, 1961; Ladeira *et al.*, 1987; George, 1993; Gutterman, 2000; Makowczynska & Andrzejewska-Golec, 2003, 2004; Budzianowska *et al.*, 2004; Li & Li, 2005a, b). These factors must be adapted to each *Plantago* species to optimize seed germination and plant growth through variations, *i.e.* pre-germination treatment, photoperiod, structure of the substrate…

In vitro cultures allow effective micropropagation in particular of *P. ovata* which presents a great economical stake for India (Pramanik *et al.*, 1994) and widely used in Egypt for his pharmacological interest (Saker & Kawashity, 1998).

These expensive optimizations are really justified for the biomass and bioactive metabolite production from *Plantago* species of economical and pharmacological great interest, i.e. *P. asiatica* (Park *et al.*, 2007), *P. major* (Poorter *et al.*, 1988; Karpilovskaia *et al.*, 1989. Li & Li, 2005a,b) and *P. ovata* (Deters *et al.*, 2005). In addition, *P. lanceolata* may be trans-

formed by *A. rhizogenes* and two phenylethanoid heterosides, *i.e. p*-coumaroyl-glucose and feruloyl-glucose are neo-synthesized and accumulated in the roots of seedlings fed with cinnamic acid (Fons *et al.*, 1998a,c, 1999).

Indeed, the polyphenol derivatives are of great interest for industrial applications (Takagaki, 2005), *i.e.* cosmetics (Proserpio & Malpede, 1995; Tanaka, 2001; Che *et al.*, 2006), cosme-nutraceuticals (Cristoni & Morazzoni, 2003; Rull Prous *et al.*, 2005; Mazzio, 2007), functional food (Osakabe, 2006; Yamada, 2007) and medicine (Ishimaru & Shimomura, 1995; Capasso *et al.*, 1997; Metz, 2000; Liu & Henkel, 2002).

Moreover, *Plantago* metabolites defining new generations of pharmacologically active compounds through biotransformation processes should definitely help fill some of the weaknesses of current therapeutic arsenal and develop it against present and future therapeutic challenges. Anyway, further investigations should be carried out to screen more *Plantago* species using the engineering *in vitro* culture methods highlighted in our 20-year review.

Table I.- Light measurement, photoperiod and relative humidity used for some experimental cultures of *Plantago* species. a: *P. arenaria* Waldst. & Kit.; c: *P. coronopus*; l: *P. lanceolata*; maj: *P. major*, mar: *P. maritima*; med: *P. media*; r: *P. rugelii* Decne.

Tableau I.- Mesure de la lumière, photopériode et humidité relative relevées pour quelques cultures expérimentales d'espèces de *Plantago*.

Light measurements		P (h)	T (°C)	RH (%)	*Plantago* species	References
	55	12	21/16	80	l	Smakman & Hofstra, 1982
	50	12	20	65	l, maj	Blacquière & de Visser, 1984
	50	12	20	60-70	l, maj	Blacquière *et al.*, 1988
Irradiance	70	14	23	70	l	Freijsen *et al.*, 1989
W.m^{-2}	35	15	25/13	70	mar, med	Harvey, 1989
	58	12	20/18	60	mar, med	Maathuis & Prins, 1990
	58	12	20/18	60	mar, med	Staal *et al.*, 1991
	80	14	23	70	l	Freijsen & Otten, 1993
	220-280	12	20	70-80	maj	Baas & Kuiper, 1989
	150	12	24/18	70	maj	Dijkstra & Kuiper, 1989
	400	14	20/15		mar	Flanagan & Jefferies, 1989
	300	12	20	80	maj	Dijkstra *et al.*, 1990
	200	16	20/15	50-60	a, c, l, med	Fitter & Stickland, 1991
	300	13	25/20		maj, r	Reekie & Bazzaz, 1992
	450	15	23/15		l, m, maj, mar, med	Reiling & Davison, 1992
Photon flux density	150	16	20	70	mar	Rozema *et al.*, 1992
µmol.m^{-2}.s^{-1}	310	16	23/17	55	med	Ferris & Taylor, 1994
or µE.m^{-2}.s^{-1}	300-330	12	20	65	maj	Fonseca *et al.*, 1997
	100	14	20/15	70	l	Poot *et al.*, 1997
	150	16	28/22	40-55	l	Schmidt *et al.*, 1997
	200	15	25/15		maj	Whitfield *et al.*, 1997
	200	14	20/15	65	maj	Lyons & Barnes, 1998
	100	16	20/15		l	Staddon *et al.*, 1998
	216	16	25/15	50-70	l	Sanderson & Elwinger, 2000a,b
	150	16	24/22	40-55	l	Schmidt & Steinbach, 2000
Illuminance	16000	14	36/20	30-100	mar	Gagnaire *et al.*, 1975
lux	9000	16	26/20	40	m	Schmidt *et al.*, 1990

Table II.- *In vitro* culture conditions of *Plantago* tissues (media, light, temperature and humidity). *culture media used for the different steps ( ).
Tableau II.- Conditions de culture *in vitro* de tissus de *Plantago* (milieu, lumière, température et humidité relative).

Species	Original tissue, steps* and tissue produced	Culture media	Culture conditions	References
P. asiatica	seeds : seedlings explants : embryogenic callus initiation callus : somatic embryogenesis and/or organogenesis	: MS + 0.2 mg.dm^{-3} Kin + 1 mg.dm^{-3} GA_3 : MS + 0.5-1 mg.dm^{-3} 2,4-D + 0.2 mg.dm^{-3} BAP : MS + various combinations of BAP with 2,4-D and NAA		Makowczynska & Andrzejewska-Golec, 2000
	: shoot-tip multiplication : rooting	: MS + 0.1 mg.dm^{-3} IAA + 1 mg.dm^{-3} BAP : MS		Makowczynska & Andrzejewska-Golec, 2003
	explants : cream-colored callus initiation explants : green-coloured callus initiation	MS + 0.5 mg.dm^{-3} 2,4-D + 0.2 mg.dm^{-3} BAP MS + 0.1 mg.dm^{-3} IAA + 2 mg.dm^{-3} BAP		Makowczynska & Andrzejewska-Golec, 2004
	: callus induction, : budding : rooting	: MS + 2,4-D : MS + BAP : MS + BAP + NAA		Tu, 1996 (Abstract)
P. lanceolata	leaf and root explants : multiplication : rooting leaf and root explants : callus initiation and growth	: MS + 11.42 µM IAA + 9.29 µM Kin : MS + 5.71 µM IAA : MS without NH_4NO_3 + 4.52 µM 2,4-D + 0.46 µM Kin	T: 25 °C, P: 16 h LM: 60 µmol.m^{-2}.s^{-1}	Budzianowska *et al.*, 2004
	seeds : seedlings	: MS	T: 25 °C, P: 12 h LM: 2000 lux	Fons *et al.*, 1998a,c
	seeds : seedlings leaf and root explants : culture of hairy roots	: MS : MS modified (N/5)	Darkness	Fons, 1998 Fons *et al.*, 1999
	hypocotyl and cotyledon explants : shoot regeneration, : micropropagation, : rooting : acclimatization	: MS + 3.33 µM BAP + 1.34 µM NAA : MS + 0.45 µM TDZ + 0.09 µM IBA : MS + 1.16 µM Kin + 0.98 µM IBA + 2.69 µM NAA : 70% sand and 30% clay mix in pots	T: 24 °C, P: 16 h LM: 40 µmol.m^{-2}.s^{-1} T: 28 °C, RH: 90% to 40%	Khawar *et al.*, 2005
P. lanceolata	embryon : callus induction	: NT, MS	Darkness, T: 25 °C	Bräutigam & Franz, 1985
P. psyllium *P. major*	leaf explants : callogenesis : bud differentiation : root induction	: MS + 0.8-1.2 mg.l^{-1} NAA : MS + 3.5-4.5 mg.l^{-1} BAP : MS/4 - MS/2	P: 10-14 h, T: 24-26 °C LM: 2500-3500 lux	Li & Li , 2005a
	seeds : adventitious bud : rooting	: MS + 0.5-4.0 mg.l^{-1} TDZ + 0.1-0.3 mg.l^{-1} IAA : MS		Li & Li , 2005b
	callus : regeneration (roots followed by shoot bud regeneration)	: MS + 1 mg.l^{-1} 2,4-D + 0.5 mg.l^{-1} BAP : MS 0.5 mg.l^{-1} BAP	T: 25 °C, P: 16 h LM: 2500 lux	Mathur *et al.*, 1991
	shoot tips : proliferation : rooting	: MS mod.+ 0.5 µM BAP : MS mod.+ 1 µM NAA	T: 24 °C, P: 16 h LM: 15 µmol.m^{-2}.s^{-1}	Mederos & Méndez, 1991 Mederos *et al.*, 1997/1998
	leaf explants : callogenesis leaf explants : caulogenesis leaf explants : rhizogenesis	: MS + 2 mg.l^{-1} 2,4-D + 0.5 mg.l^{-1} Kin : MS + 0.5 mg.l^{-1} IAA + 1mg.l^{-1} Kin : MS + 2 mg.l^{-1} IBA + 1 mg.l^{-1} NAA	T: 25 °C, P: 16 h	Saker & Kawashity, 1998

	shoot apices : shoot induction : rooting	: MS + 4.6 µM Kin + 0.05 µM NAA : MS/2 + 5 µM IBA + 0.05 µM Kin	T: 25 °C, RH: 55% P: 16 h, LM: 30 $\mu E.m^{-2}.s^{-1}$	Barna & Wakhlu, 1988
	hypocotyl : callus induction : shoot regeneration : rooting	: MS + 4.5 µM 2,4-D + 4.6 µM Kin : MS + 0.05 µM NAA + 18.4 µM Kin : MS/2 + 5 µM IBA	T: 25 °C, RH: 50-55% P: 16 h LM: 6 $\mu mol.m^{-2}.s^{-1}$; 30 $\mu mol.m^{-2}.s^{-1}$	Wakhlu & Barna, 1988, 1989
	hypocotyl : callus induction : root formation followed by shoot bud induction : root induction	: MS + 4.5 µM 2,4-D + 4.6 µM Kin : MS + 11.4 µM IAA + 0.9 µM Kin : MS/2 + 5 µM IBA	T: 25 °C, P: 16 h LM :30 $\mu E.m^{-2}.s^{-1}$	Barna & Wakhlu, 1989
P. ovata	seed : leaf segments callus induction, cell suspension or embryogenesis : germination and seedlings formation	: MS : MS+ 1 µM 2,4-D + 2 µM Kin : MS + 4.6 µM Kin	T: 24 °C, P: 16 h	Jasrai *et al.*, 1993
	shoot buds : callus initiation or : somatic embryogenesis	: MS + 4.5 µM 2,4-D + 2.3 µM Kin : MS + 4.4 µM BAP + 2.7 µM NAA +2 $g.l^{-1}$ CH : MS+ 4.4 µM BAP + 2.7 µM NAA + 10% CW	T: 25 °C, P: 16 h	Das & Sen Raychaudhuri, 2001
	hypocotyl :c allus induction : shoot regeneration : rooting	:MS + 0.4 $mg.l^{-1}$ NAA + 0.4 $mg.l^{-1}$ BAP : MS + 0.2 $mg.l^{-1}$ IAA + 5 $mg.l^{-1}$ BAP : MS	T: 22-25 °C, P: 16 h RH: 55-60% LM: 1500 lux	Pramanik *et al.*, 1994, 1996
	shoot tips : multiplication : rooting	: MS + 0.2 $mg.l^{-1}$ IAA + 5 $mg.l^{-1}$ BAP/Kin : MS		Pramanik *et al.*, 1995

Table III.- Culture conditions of *Plantago* species (light, temperature, relative humidity and nutrition).
Tableau III.- Conditions de culture d'espèces de *Plantago* (lumière, température, humidité relative et nutrition).

Plantago species	Original tissue	Media	Conditions	References
P. lanceolata	seeds/plants	Sand + soil, Hewitt solution	P: 18h	Newman & Rovira, 1975
		Pots + CO_2 Vermiculite-filled, Soil/Turface®/sand (2:1:5)	P: 14/16 h, T: 25 °C, RH: 70%	Fajer *et al.*, 1991
		Petri dishes cotton wool pads, Nutr. Sol.	Natural light	Horner & Bell, 1995
		Silica gravel washed graded autoclaved Long Ashton mod.	P: 16 h, T: 15, 21, 27 °C, RH: 75%	Forbes *et al*, 1996
		Sand, CO_2 + mycorrhize	Natural photoperiod	Rouhier & Read, 1998
		Nutr. Sol.	GB in the light	Freijsen *et al.*, 1989
		Petri dishes, sand/hydroculture, Nutr. Sol.	P: 14 h, T: 20/15 °C, RH: 70%	Poot *et al.*, 1997
		GB, Nutr. Sol.	P: 14 h, T: 23 °C, RH: 70%	Freijsen & Otten, 1987, 1993
		Pots, Nutr. Sol.		Verhagen *et al.*, 1995
		Quartz sand, Nutr. Sol. + Cu or Fe	P: 16 h, T: 28/22 °C, RH: 60%	Schmidt *et al.*, 1997
		Sterilized vermiculite Hoagland mod., Lewis and Powers mod.	1) P: 14 h, T: 25/20 °C 2) P: 12 h T: 21/16 °C, RH: 80%	Smakman & Hofstra, 1982
		Poots, Hoagland mod.	Greenhouse	Eissenstat, 1990
		Petri dishes, glass beads, Hoagland mod.	P: 14 h, T: 22/15 °C, RH: 70%	Van Hinsberg, 1997 Van Hinsberg & Van Tienderen, 1997
		Filter paper, Pots Sand/Terragreen® (1:1) + P	P: 16 h, T: 20/15 °C	Staddon *et al.*, 1998
	plants	Nut. sol. + Fe	P: 16 h, T: 26/20 °C, RH : 40%	Schmidt *et al.*, 1990
P. major	pollen	Kozar (1974)	T: 20 °C	Ayyad & Baka, 1993
	seeds/plants	Sandy soil Nutr. Sol. + P + inoculum	P: 12 h, T: 20 °C RH: 70-80%	Baas & Kuiper, 1989
		Petri dishes/Pot, Standard potting compost + O_3	P: 15 h, T: 23/15 °C	Reiling & Davison, 1992
		Standard John Innes n° 2 potting compost	Controlled-environment chambers + O_3 P: 15 h, T: 25/15 °C	Whitfield *et al.*, 1997
		Standard John Innes n° 2 potting compost, seed trays/ plugs, modules, Pots + O_3	P: 14 h, T: 20/15 °C, RH: 65%	Lyons & Barnes, 1998
		Commercial soil mixture (Biomix Super); 6-BAP, NAA GA_3…	P: 12 h, T: 24/18 °C, RH: 60-80%	Dijkstra & Kuiper, 1989
		Sterilized soil Nutr. Sol., Hoagland mod.+ CO_2	P: 12 h, T: 20 °C, RH: 65%	Den Hertog *et al.*, 1996 Fonseca *et al.*, 1997
		Vermiculite-container, Hoagland mod., pH 6, GA_3	P: 12 h, T: 20 °C, RH: 70-90%	Dijkstra *et al.*, 1990, Dijkstra & Lambers, 1989
	seeds	Vermiculite, Hoagland	Natural light, T: 27/23 °C	Miao *et al.*, 1991
	plants	Nutr. Sol. + BAP	P: 12 h, T: 20 °C, RH: 60%	Kuiper *et al.*, 1991

	seeds	Petri dishes, filter paper	Darkness, T: 22 °C	Siegel & Siegel, 1975
P. maritima	seeds/plants	Hydroculture Hoagland + Boron Petri dishes, paper filter, Hoagland Sol. Pots Standard soil/silica sand + 10% nutrient solution (Johnson *et al.*, 1957)	pH: 6.5, P: 16 h, T: 20 °C, RH: 70% pH: 4.5, P: 14 h, T: 36/20 °C, RH: 30-100% P: 14 h, T: 20/15 °C	Rozema *et a.l*, 1992 Gagnaire *et al.*, 1975 Flanagan & Jefferies, 1989
P. media	seeds/plants	Vermiculite, sol. of Liquinure (Fisons Plc., Ipswich)	P: 16 h, T: 23/17 °C, RH: 55%	Ferris & Taylor, 1994
P. lanceolata	seeds/plants	Vermiculite, Nutr. Sol.	P: 12-14 h, T: 22/16 °C, RH: 50-65%	Blacquière *et al.*, 1988
P. major	plants	Nutr. Sol.	P: 12 h, T: 20 °C, R: 65%	Blacquière & de Visser, 1984
P. lanceolata *P. maritima*	seeds/plants	Sand, Nutr. Sol.(New Jersey) + NaCl	P: 16 h, T: 25/15 °C, RH: 80-90%	Ferron *et al.*, 1977
P. lanceolata *P. media*	seeds	Soil	P: 12 h or dark, T: 22/12 °C	Pons, 1991a
P. major *P. rugelii*	seeds/plants	Vermiculite, Turface®, sand, Hoagland mod.	P: 13 h, T:25/20 °C	Reekie & Bazzaz, 1992
P. maritima *P. media*	seeds/plants	Vermiculite/containers,Hoagland mod + NaCl Sand, Nutr. Sol. + NaCl	P: 12 h, T: 20/18 °C, RH: 60% P: 15 h, T: 25/13 °C, RH: 70%	Maathuis & Prins, 1990 Staal *et al.*, 1991 Harvey, 1989
P. arenaria *P. coronopus* *P. lanceolata* *P. media*	seeds/plants	Filter paper, Pot, Rorison Nutr. Sol. (Hewitt, 1966)	P: 16 h, T: 20/15 °C, RH: 50-60%	Fitter & Stickland, 1991
P. coronopus *P. lanceolata* *P. major* *P. media*	seeds/plants	Vermiculite, Hoagland mod.	P: 13 h, T: 20/16 °C, RH: 60-80%	Smit & Woldendorp, 1981
P. lanceolata *P. major* *P. maritima* *P. media*	plants	Quartz sand, Pot, Nutr. Sol. + NaCl	open air, mobile roof	Königshofer, 1983

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