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Research review paper

Phaseolus vulgaris — Recalcitrant potential

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ABSTRACT

Since the ability to genetically engineer plants was established, researchers have modified a great number of plant species to satisfy agricultural, horticultural, industrial, medicinal or veterinary requirements. Almost thirty years after the first approaches to the genetic modification of pulse crops, it is possible to transform many grain legumes. However, one of the most important species for human nutrition, *Phaseolus vulgaris*, still lacks some practical tools for genomic research, such as routine genetic transformation. Its recalcitrance towards *in vitro* regeneration and rooting significantly hampers the possibilities of improvement of the common bean that suffers from many biotic and abiotic constraints. Thus, an efficient and reproducible system for regeneration of a whole plant is desired. Although noticeable progress has been made, the rate of recovery of transgenic lines is still low. Here, the current status of tissue culture and recent progress in transformation methodology are presented. Some major challenges and obstacles are discussed and some examples of their solutions are presented.

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Introduction

Legumes, the third largest family of higher plants, are notoriously recalcitrant both to regeneration and transformation. Grain legumes (that rank third behind cereals and oilseeds in world production) have lower responsiveness to *in vitro* regeneration compared to the forage legumes (Veltcheva and Svetleva, 2005). This is also the case for *Phaseolus vulgaris*, an economically important crop. The common bean is the most important food legume for direct human consumption in several countries of Latin America and Africa, however its position cannot be overestimated in the USA, Canada or India. Even the Common Market of the European Union, focused rather on cereals, admits to cropping more than 1300 its varieties specified in the Common Catalogue of Varieties of Vegetable Species (2011) (including dwarf and climbing ones). It seems completely justified as beans combined with cereals assure a balanced diet of energy and protein. Bean seeds provide important minerals, vitamins, dietetic fibre but no unsaturated fatty acids (De LaFuente et al., 2011).

As *P. vulgaris* represents a major protein source in the population's diet, it is obvious that it is still of high agronomic interest worldwide. Among over 30 species of the genus *Phaseolus* (according to different

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authors it is difficult to estimate how many *Phaseolus* species exist, the number may reach even 50 or 60 species) the common bean is the most widely distributed crop, occupying more than 90% of the area intended for beans in general (Broughton et al., 2003; Morales, 2006). Having been adapted to diverse environmental conditions, the common bean is not free from biotic and abiotic constraints. It suffers from six widespread major diseases and some unfriendly abiotic conditions such as soil toxicities, drought stress or nutritional deficiencies (Beaver and Osorno, 2009; Popelka et al., 2004). It is a challenge on which both plant biotechnology and conventional breeding methods have been focused on legume improvement for several years. And there may be many targets for such improvement. Apart from the above, they may concern for example the enrichment of the seed proteins of pulse crops in sulphur-containing amino acids, changing the plant anatomy or reducing the time needed for flowering and seed setting in long duration crops (Eapen, 2008). Also the usage of legumes as 'green factories' seems completely justified.

Consequently, several international initiatives (the Medicago Genome Consortium; International Conferences on Legume Genomics and Genetics ICLGG) that concentrate primarily on the field of legume genomics and genetics (Colpaert et al., 2008). For *P. vulgaris* studies, an international consortium — '*Phaseomics*' was established in 2000 in Sevilla, Spain (Broughton and Aguilar, 2005). The main purpose of this initiative was to establish the necessary framework of knowledge for the advancement in studies of bean. *Phaseomics* gathers a number of scientists from all over the world that focus on different aspects of widely understood *Phaseolus* biology. Due to these efforts plant regeneration and transformation in the legume family have been achieved for several species, however one of the most important food legumes, *P. vulgaris*, remains recalcitrant to both routine *in vitro* breeding and genetic engineering. It is still difficult to determine whether beans are generally not amenable to regeneration or transformation only because of their indigenous lack of competence or how to crush their resistance simply remains undiscovered. However, in the 1970s and 1980s, a similar situation existed regarding cereals, that were considered to have low potential for regeneration and transformation processes and then the concentrated efforts of plant scientists enabled success in the field of cereal engineering (Shrawat and Lorz, 2006). The number of researchers interested in legume biology and the undoubtedly observed dynamic development of knowledge, justify the opinion that also in the case of *Phaseolus* it is a question of the time to devise repeatable and efficient procedures.

Some recent and promising reports, both on regeneration and transformation protocols, are presented here. Whether any of them may become a base for the routinely used procedure is open to question. It should be pointed out that the reported outcomes are presented rather in the form of confrontation among the trends in *P. vulgaris* research than of direct comparisons.

Regeneration

Tissue culture of *P. vulgaris* is repeatedly considered to be difficult. It is particularly inconvenient as the lack of a rapid and efficient regeneration system hampers possibilities of its genetic improvement. Although the number of papers is available, the proposed methods of regeneration still seem to be not easily reproducible. The utility of the tissue culture achievements established for other representatives of the *Phaseolus* genus also seems rather exaggerated. For example, application of the procedures successfully used for regeneration of whole plants of different *Phaseolus* species (*Phaseolus acutifolius*, *Phaseolus coccineus*, *Phaseolus polyanthus*) results only in shoot production in the case of *P. vulgaris* (Delgado-Sánchez et al., 2006). That suggests at least a species-specific protocol. Apart from the physiological state of the explant, cell or tissue specialisation of the culture and cultivation conditions, a plant genotype is the basic factor responsible for regeneration processes (Svetleva et al., 2003). Thus, genotype limitations indirectly underlie the difficulty in development of routine regeneration procedure for legumes or even beans. It is unquestionable that beans demonstrate extremely high diversity regarding regeneration responsiveness. All three classic pathways of *in vitro* propagation (organogenesis, somatic embryogenesis and proliferation of shoot meristems from the regions surrounding the shoot bud) were described for the common bean with limited efficiency and low repeatability. In consequence the necessity of a genotype-dependent and cultivar-specific procedure is suggested.

Most of the published procedures were based on direct organogenesis or shoot development from meristematic cells (Arellano et al., 2009). Many examples of a direct organogenesis pathway may be found in the literature e.g. reported by Ahmed et al. (1998), Ahmed et al. (2002), Albino et al. (2005), Ebida (1996), McClean and Grafton (1989), Mohamed et al. (1992) or Quintero-Jiménez et al. (2010). Yet, to the best of our knowledge and belief, there are only a few protocols based on indirect organogenic regeneration of the common bean (Arellano et al., 2009; Collado et al., 2013; Mohamed et al., 1993; Zambre et al., 1998) as in the case of induction of somatic embryogenesis it occurs rather sporadically (Jacobsen, 1999; Kwapata et al., 2010; Martins and Sondahl, 1984; Nafie et al., 2013). Nevertheless, until now several types of cells, tissues and organs (cotyledonary nodes, embryonic axes, auxiliary shoots, cotyledon with split embryo axis, internodes, hypocotyls, leaves, leaf petioles or intact seedlings) have been used to induce all the regeneration pathways (Albino et al., 2005; Delgado-Sánchez et al., 2006; Franklin et al., 1991; Mahamune et al., 2011; Thảo et al., 2013). However, it should be noted that the protocols named did not always yield regeneration of the whole *P. vulgaris* plants.

The number of published regeneration procedures of common bean is quite enormous and the reported approaches cover all pathways of *in vitro* regeneration: organogenesis, somatic embryogenesis and proliferation of shoot meristems from the regions surrounding the shoot bud (Eapen, 2008). Such a general description of regeneration can be found in the literature. However, the above depiction of it is very wide and describes each way of plant development except natural morphogenesis of a generative origin. From this point of view, it should be rather characterized as the ways in which plants can be propagated through tissue culture. Thus, it is very important to keep it in mind that in tissue culture practice especially focused on plant transformation, the true term "regeneration" functions in a narrower context. Regeneration comprises plant development from somatic tissue section lacking preformed meristems (i.e. leaf, calluses) while proliferation/micropropagation occurs using meristematic tissues like axillary bud regions. Plant regeneration itself can occur by two pathways: organogenesis or somatic embryogenesis (Phillips and Hubstenberger, 1995). At this level it is also very important to make a clear distinction between wider terms – organogenesis and somatic embryogenesis – and their subtypes: direct (adventitious) and indirect (de novo origin *via* i.e. callus) processes. As these terms are used in many different ways in the literature their precise usage in the scientific reports would greatly simplify the comparison of the results and determination of the current status of the research on the regeneration of *P. vulgaris* plants.

Here we present the examples of different approaches to common bean regeneration and attempt to refer to the main problems discussing the ways they have been solved.

Obstacles and solutions in regeneration protocols

It may be concluded from scientific reports that some obstacles in the regeneration process have been identified and currently efforts are made to eliminate them. According to many authors it is possible to influence *the plant competency* (Cruze de Carvalho et al., 2000; Mohamed et al., 1992; Veltcheva and Svetleva, 2005; Zhang et al., 1997). *Precultivation* of parent plants on a medium enriched with BAP (benzylaminopurine), TDZ (thidiazuron) or CPPU (forchlorfenuron) may stimulate the division of competent cells and indirectly influence