

## S U M M A R Y

### OBTAINING AND GENOTYPE INVESTIGATIONS OF ANDROGENIC HAPLOIDS FROM BULGARIAN PEPPER (*CAPSICUM ANNUUM* L.) VARIETIES

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In the dissertation are included seven year results from our studies.

The aim of the Ph.D. thesis was: to characterize androgenic capacity of Bulgarian pepper genotypes in anther culture, as well as to establish suitable nutrient media for microspore-embryogenesis induction and optimal variant of low-temperature pretreatment of flower buds; to investigate the pepper genome for presence of *BABY BOOM*- and *LEAFY COTYLEDON* DNA-sequences.

There were investigated 21 sweet pepper (*Capsicum annuum* L.) selection lines, varieties and hybrids, representative sort-types blocky-type, kapiya, conicum and red pepper for grind (paprika), as well the anther culture's response on 16 nutrient media variants. In the present work was established the prevalence of microspores in late uninuclear phase (suitable for androgenic induction) in anthers isolated from 4-4.5 mm long flower buds. The explants from all genotypes produced direct embryoids or nonembryogenic calluses. Indirect organogenesis showed only line 145 and variety "Zlaten medal". The highest frequency of direct embryogenesis was established in lines 145 and 1312, variety "Albena" and hybrid 50/01. Development of embryoids to regenerated plants in two lines (145 and 1957), four varieties ("Kurtovska kapiya", "Zlaten medal", "Stryama" and "Hebur") and two hybrids (50/01 and 91/01) were observed. The lines and varieties were most suitable donors for anther culture as well as blocky-type peppers. The donor plant's genotype greatly influenced over induction of direct embryogenesis, embryo conversion into plants and *in vivo* adaptation. The induction nutrient media C (Dumas de Vaultx et al., 1981), Cm (Sibi et al., 1979) and MS-1 (Matsubara et al., 1998) were suitable for productive anther culture. There was established embryo formation on media C only after 40-days cultivation on the same media. The anthers cultivated 12-days on C-media and transferred on media for regeneration R (by Dumas de Vaultx et al., 1981) did not develop embryos. Five combinations of media for induction and regeneration (C-0 > R; MS-3 > MS; C > R; C+ > R and MS-1 > MS) provoked embryo conversion into plants and were suitable for the aim of anther culture. The silver nitrate (5 mg/l) added to media C and Cm do not enhance embryogenesis, but in MS-3 stimulated embryo formation. Probably the effect of silver nitrate on anther culture response depends of complete composition of nutrient media and applied growth regulators. It was established that low-temperature pretreatment (4° C) of flower buds for 24 h (for varieties and sort-type conicum) and 48 h (for breeding lines, hybrids, sort-types conicum and kapiya) stimulate direct embryogenesis. The low-temperature pretreatment has no positive effect on embryogenesis in blocky-type pepper and red pepper for grind (paprika). The more prolonged cold treatment (72- and 96 h) has no positive effect in all studied genotypes with except of "Albena" variety (96 h stimulate embryogenesis). It was established early

spontaneous diploidization of the genome for the great part of obtained regenerants (93.75 %) that resulted in haploid-diploid chimeras.

*BABY BOOM* (*BBM*) was the first gene identified to be involved in switch to androgenesis and initiation of embryogenesis in microspore culture (Boutilier et al., 2002; Maraschin et al., 2005; Malik et al., 2006, 2007). *LEAFY COTYLEDON1* (*LEC1*) and *LEAFY COTYLEDON2* (*LEC2*) genes are involved in conferment of embryogenic competence in cells (Lotan et al., 1998; Harada, 2001) and are expressed in early microspore embryogenesis in *Brassica napus* (Malik et al., 2006, 2007). No one of these genes has been yet found in pepper. The aim of this study was to search for *BBM*- and *LEC*-like sequences in sweet pepper genome. We looked for *BBM*- and *LEC*- nucleotide sequences in NCBI databases. Multiple alignments of homologous sequences were performed using Vector NTI 10.1 softwer. Established conserved consensus areas in sequences from different plant species serve for disain of degenerative primers (two pairs for each of genes). PCRs were carried out with pepper genome DNA and amplified products were isolated, cloned and sequenced. The nblast in NCBI database display high similarity between putative *BBM*-like sequence from pepper and annotated *BBM* sequences: *Arabidopsis thaliana* *BABY BOOM* (*BBM*) gene (AF317905.1) (identity 84 % - 88 %); *Brassica napus* AP2/EREBP transcription factor *BABY BOOM1* (*BBM1*) gene (AF317906.1) (identity 82 % - 88 %); *Medicago truncatula* AP2/EREBP transcription factor *BABY BOOM* (*BBM*) (AY899909.1) (80 % - 85 %); *Brassica napus* AP2/EREBP transcription factor *BABY BOOM2* (*BBM2*) gene (AF317905.1) (78 % - 80 %), etc. The pepper's sequence don't show similarity with *Capsicum annuum* putative AP2/EREBP transcription factor (DQ266257, DQ247966 и DQ249815). It was established high similarity between putative *LEC*-like sequence from pepper and annotated *LEC* sequences: *A. thaliana* *LEC1* (*LEAFY COTYLEDON1*) transcription factor (NM\_102046.4) (identity 83 %); *Kalanchoe marmorata* *LEC1*-like gene (EF605509.1) (identity 82 %); *Bryophyllum delagoense* *LEC1*-like gene (EF605510.1) (82 %); *Oryza sativa* HAP3 transcriptional activator (*LEC1A*) (AY062183.1) (82 %); *Theobroma cacao* *LEC1*-like gene (AM494833.1) (82 %); *Zea mays* *Lec1* (NM\_001112048.1; AF410176.1; AF410176) (81 %); *Kalanchoe tomentose* *LEC1*-like gene (EF605519.1) (81 %); *Oryza sativa* *Lec1* gene (AY264284.1) (80 %); *Helianthus annuus* l1l gene for leafy cotyledon 1-like protein (AJ879074.1) (79 %), etc. The obtained *BBM*-like and *LEC*-like sequences from pepper were subjected to bioinformatics analysis for determining exon-intron structure. Exons were translated in-silico in proteins and protein blast in NCBI display presence of AP2-type functional domains in putative *BBM* from pepper – typicals for *Arabidopsis* and *Brassica* *BBM* proteins. The putative *LEC* from pepper carried Cbfd-NFYB-HMF motive presented in: *A. thaliana* Leafy cotyledon1 transcription factor (NP\_173616.2); *Zea mays* *Lec1* (NP\_001105518.1); *Oryza sativa* *Lec1* (NP\_001047986.1) etc.

This is the first report of existence of *BBM*-like and *LEC*-like sequences in the genome of *Capsicum annuum*.