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Doubled Haploid Breeding Technology for Pepper

Towards a generic protocol for doubled haploid plant production and development of tools to predict cultivar response

Doubled Haploid technology speeds up plant breeding programs by many years

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Partner's elite cultivars → Technology → Trait selection + plant breeding → New elite hybrids with enhanced

- Yield potential
- Size
- Quality
- Metabolites
- Health
- Extreme weather Adaptation
- Disease and crop protection

DH technology is a non-GMO breeding tool that creates full inbred plants in one generation which are used to create elite hybrids

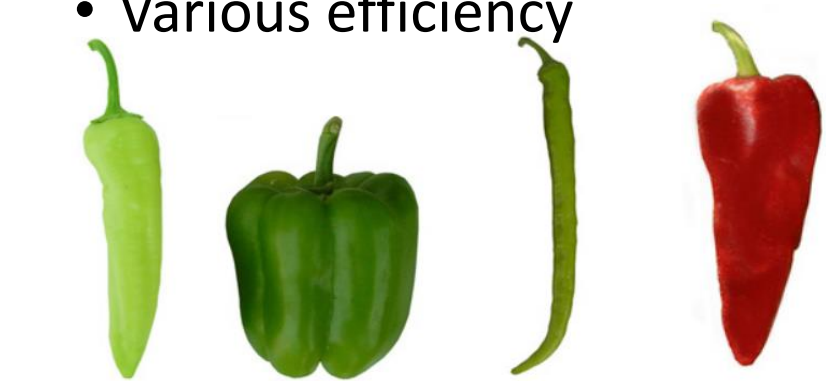
Fytagoras' goal

Providing doubled haploid technology service to customers around the world

- Reliable protocol for microspore regeneration
- Accurate risk and cost estimation
- Cost effective DH plant production

Current challenges for a generic Capsicum protocol

- **Genetic variability in response:**
  - Towards different protocols (microspore, anther, shed)
  - Induction efficiency
  - Embryo quality
  - Green plant conversion
  - Doubled haploidy rate
  - Various efficiency



Pictures from Davut Keles et.al. 2015, Hort Science 50 (11), 1671-1676

Table 1. Plant, haploid, diploid plant number, and spontaneous double haploidy rate in different pepper types.

Species types	Anther no.	P no.	HP no.	DP no.	SDH %[z]
Green type-1	300	5	4	1	20.0
Green type-2	300	20	13	5	25.0
Green type-3	300	11	10	1	9.1
Green type-4	300	17	12	4	23.5
Green type-5	300	16	12	4	25.0
Green type-6	300	12	11	1	8.3
Green type-7	300	9	5	4	44.4
Green type	Mean	12.9	9.6	2.9	22.2 b
Charleston type-1	300	18	10	8	44.4
Charleston type-2	300	14	8	6	42.9
Charleston type-3	300	21	18	3	14.3
Charleston type-4	300	7	4	3	42.9
Charleston type-5	300	20	16	4	20.0
Charleston type-6	300	20	19	4	20.0
Charleston type-7	300	31	19	12	38.7
Charleston type	Mean	18.7	13.4	5.7	31.9 b
Capia type-1	300	42	32	10	23.8
Capia type-2	300	23	16	7	30.4
Capia type-3	300	33	23	10	30.3
Capia type-4	300	8	5	3	37.5
Capia type-5	300	22	14	6	27.3
Capia type-6	300	18	12	6	33.3
Capia type-7	300	11	8	3	27.3
Capia type-8	300	24	16	8	33.3
Capia type	Mean	22.6	15.8	6.6	30.4 b
Bell type-1	300	36	17	19	52.8
Bell type-2	300	51	24	27	52.9
Bell type-3	300	17	8	9	52.9
Bell type-4	300	20	12	8	40.0
Bell type-5	300	60	23	37	61.7
Bell type-6	300	25	10	15	60.0
Bell type	Mean	34.8	15.7	19.2	53.4 a
$P > f$	0.0110	0.2782	0.0001	0.0002	0.0002
$D_{5\%}$					8.913

[z]SDH was analyzed with arcsin transformed data.

P no. = Plant number obtained from anther culture; HP no. = haploid plant number; DP no. = diploid plant number; SDH % = spontaneous double haploidy rate.

Davus Keles et al., 2015, HortScience 50 (11): 1671-1676

Fytagoras' approach to a generic Capsicum protocol

Optimization of Fytagoras' protocol on microspore regeneration

- Screen response with (commercial) varieties
- **Protocol development**:
  - Plant growing conditions
  - Bud, anther and cell stage
  - Anther and cell pre-treatment
  - Media compositions
  - Cell culture
  - Timing of events
  - Evaluation + feedback

plant growing conditions	bud anther cell stage	anther cell treatment	media	cell culture	timing	evaluation feedback

Protocol development

- Plant growing conditions; relation environment and responsiveness

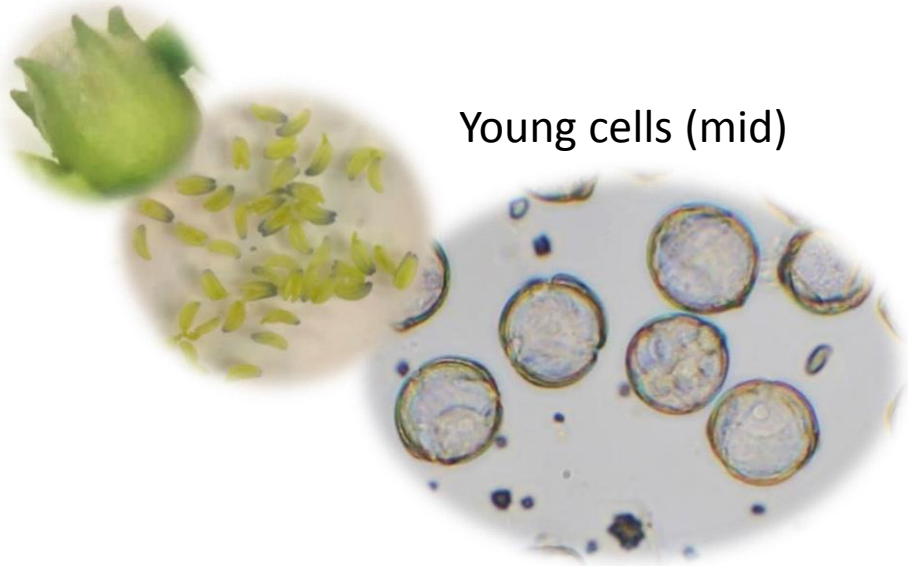
Plants are grown in climate rooms on rockwool slabs with liquid nutrition.

plant growing conditions	bud anther cell stage	anther cell treatment	media	cell culture	timing	evaluation feedback

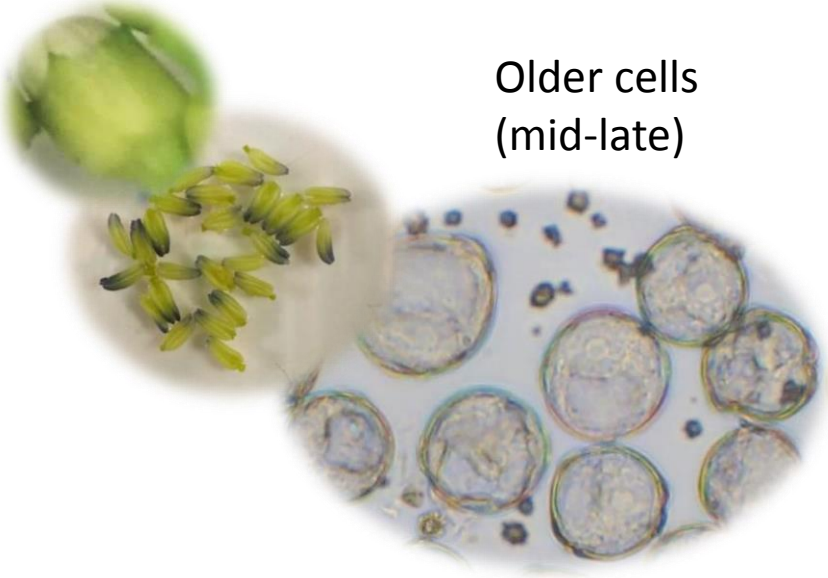
Protocol development

- Bud, anther and cell stage

Cell type influences outcome



Young cells (mid)



Older cells (mid-late)

plant growing conditions	**bud anther cell stage**	anther cell treatment	media	cell culture	timing	evaluation feedback

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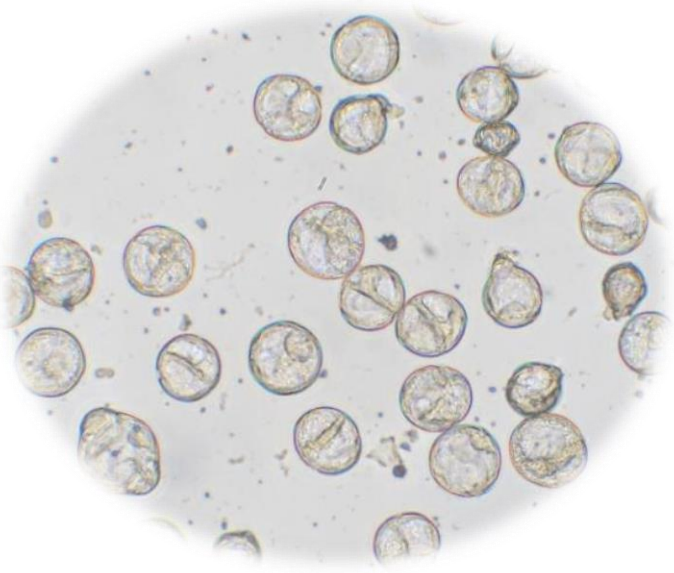
Protocol development

- Anther and/or cell pre-treatment

- Heat
- Cold
- Starvation
- Inducers

→

- Divisions
- Cell viability



Induced cell division

plant growing conditions	bud anther cell stage	**anther cell treatment**	media	cell culture	timing	evaluation feedback

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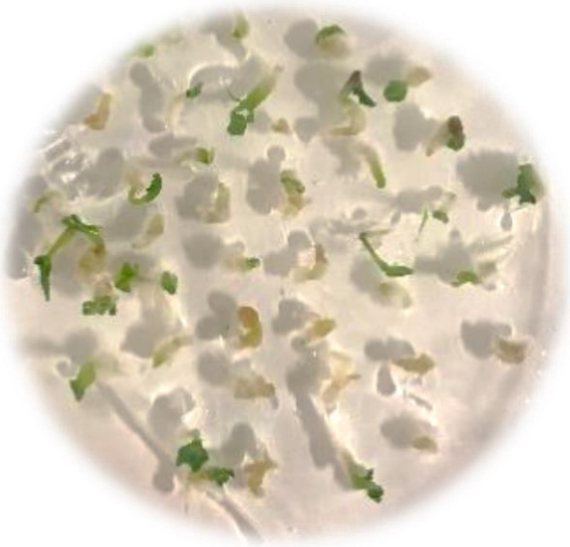
Protocol development

- Media compositions

- Micro and macro nutrients
- Sugar source
- Nitrogen source
- Hormones
- Matrices of above

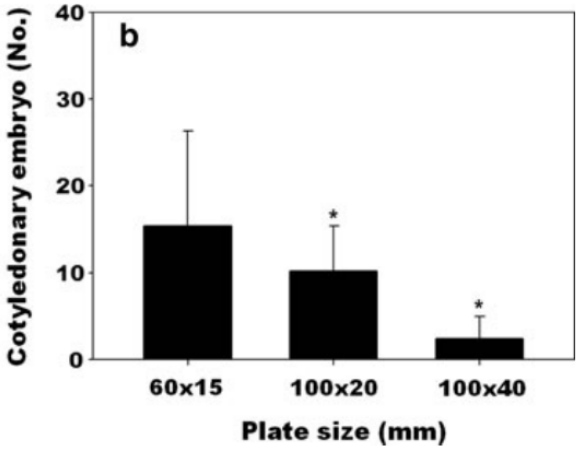


Germinating embryo's

Protocol development

- Cell culture

- Cell density
  - cells per ml
  - cells per area
  - volume-area ratio
- Culture dish size
- Culture temperature
- Co-culture
- Dual layer
- Gentle shaking



Moonza Kim, et al., Plant Cell Tiss Organ Cult (2013) 112:191–201

plant growing conditions	bud anther cell stage	anther cell treatment	media	**cell culture**	timing	evaluation feedback

Protocol development

- Timing of events
  - Pre-treatment duration
  - Medium transfer
  - Embryo differentiation
  - Embryo-plantlet conversion

cotyledonary embryo

no cotyledon embryo

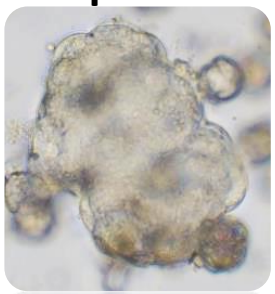
radicle only embryo

plant growing conditions	bud anther cell stage	anther cell treatment	media	cell culture	**timing**	evaluation feedback

Protocol development

- Evaluation
  - Cell morphology
  - Vital cells %
  - Induction %
  - MCS morphology + number
  - Embryo classes
  - Green plantlet morphology and conversion rate
  - Ploidy
- Feed back

compact MCS



callus MCS

Plantlet classification

Normal

Dwarf

No leaf

One leaf

Trumpet

Callus

plant growing conditions	bud anther cell stage	anther cell treatment	media	cell culture	timing	**evaluation feedback**

Fytagoras' approach to predicting variety response

Not all genotypes respond the same

→ **Aim**: predict the effectiveness and assess the risks and costs for the customer.

Prediction of variety response to Doubled Haploid technology

Early embryogenic response marker:

- Seed markers
- Plant markers
- Bud/flower markers
- Anther markers
- Cell markers

Fytagoras team working on DH related technologies.

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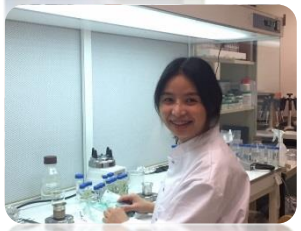

Bert van Duijn
CSO

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Thank you

Questions ??

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