INRES
Institut für Nutzpflanzenwissenschaften und Ressourcenschutz

UNIVERSITÄT BONN

Advances in Plant Breeding Methodology

Can Allohexaploid Brassicas Shows Drought Tolerant Morphological Traits ?

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Group (2)

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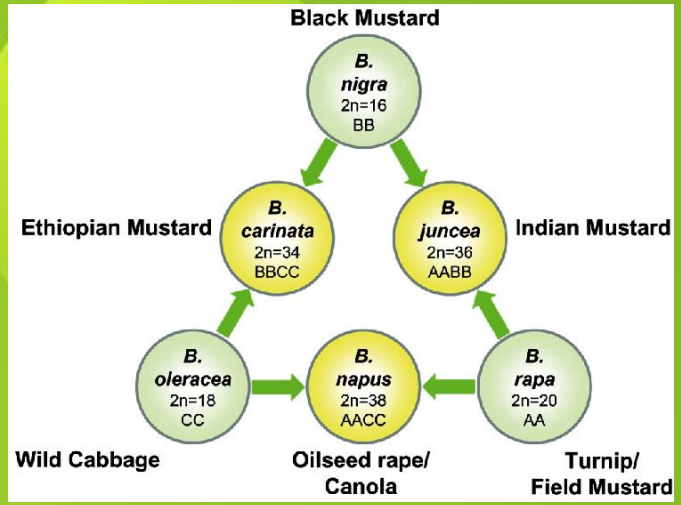
Introduction

- Family- Brassicaceae
- Important Agricultural & Horticultural Crop
- Diverse Growth habitat (Annual or Biennials)
- Developed in area with high rainfall
- Growth and seed yield production have greatly decreased owing to drought condition

Brassica U Triangle

- Three diploid species : *B.rapa* (AA), *B.nigra* (BB), *B.oleracea* (CC)
- Three allotetraploid species : *B.juncea* (AABB), *B.carinata* (BBCC), *B.napus* (AACC)



https://www.researchgate.net/figure/U-triangle-showing-the-affiliations-among-different-Brassica-species-UN-1935_fig5_224875327

Brassicas Project

Prof. Dr. Annaliese Mason

"Stabilising autopolyploid meiosis for enhanced yield,"

Brassicas Project

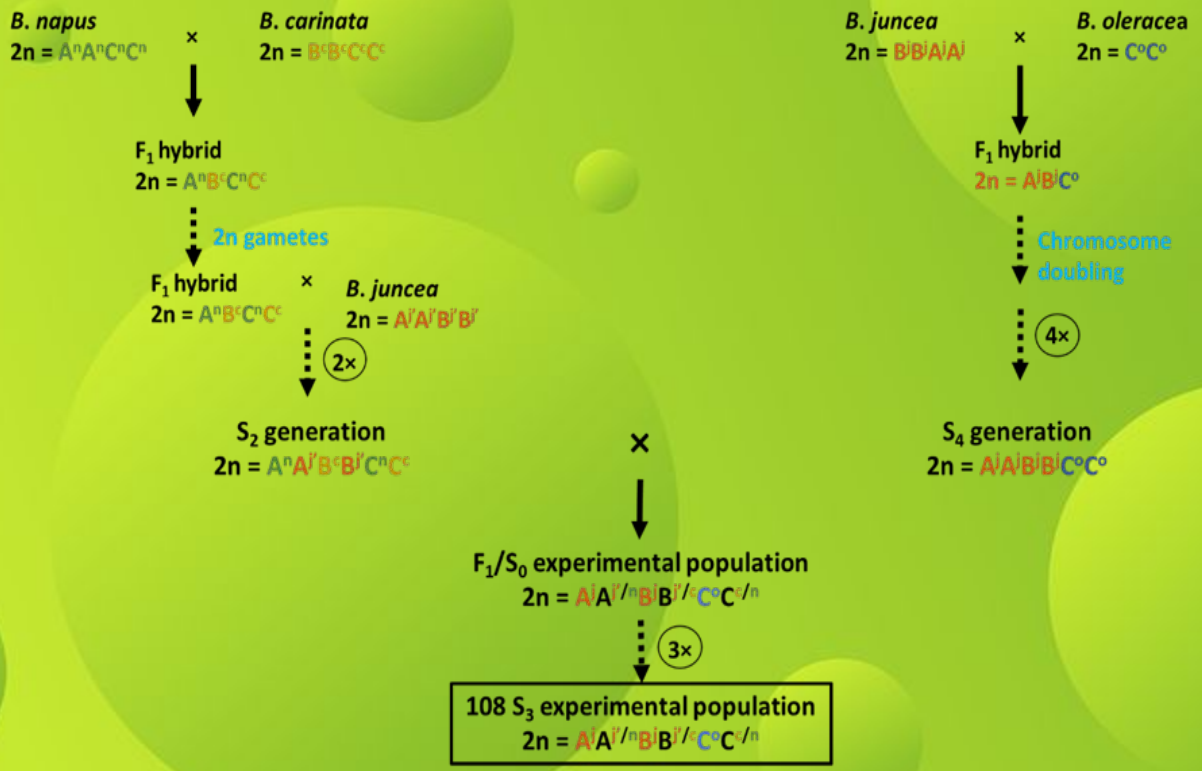


Figure 1: Production of third generation allohexaploid (AABBCC) plants through crossing between the parental *Brassica napus*, *carinata*, *juncea* and *oleracea* genotypes followed by three selfing generations of the F_1 hybrid

Maternal N6C2J2 plants

- *B.napus* (2n=AACC) * *B.carinata* (2n=BBCC) = hybrids with genome complement 2n=CCAB
- Generated gametes were crossed with *B.juncea* (AABB) = allohexaploid population
- Self - pollinating for two generations = maternal N6C2J2 plants

Paternal J301 plants

- *B.juncea* (AABB) * *B.oleracea* (CC) hand - pollination
- Ovule culture
- Colchicine treated to double their chromosome number
- Self - pollination for four generations
- Paternal J301 allohexaploid population

F1 Generation

- Crossing of maternal and paternal allohexaploid populations = N6C2J2.J301 F1 plants
- Self - pollination of F1 → generation of seeds = S1 population
- Germination with parental control seeds in March 2021 to grow until maturity
- Three selfing generations of F1 hybrid = S3 experimental population

Drought Tolerant Morphological Traits

Our Hypothesis

- Dwarf Individuals
- Small leaf area and thickness

(Many more but not analysed by us).

★ New Phytologist (2021) 231: 601–616 doi: 10.1111/nph.16996

★ https://www.researchgate.net/publication/283109457_Physiological_traits_related_to_drought_tolerance_in_Brassica

Material and Method

- Growing Date - 22.03.2023
- Location - Greenhouse (Katzenburweg 11, tunnels) and Lab- (Kirschallee 1, lab 1.045 first floor)
- Plant Layout - RCB Design (Randomized Complete Block Design)

Used Brassica hexaploids genotypes

- H29-6 (1-12) (12x)
- H58- 9 [individual 7 to individual 12] (6x)
- H12-1 [individual 1 to individual 7] (7x)
- Controls-> J2 (1-3) (3x) and C1 (1-3) (3x)

Data Collection

Phenotyping

- Rosette Diameter (cm)
- Bolting Height (cm)
- Plant Height(First Flowering stage) (cm)
- Day of First Flowering
- Pollen Viability Percentage
- Leaf(Length & Width) (cm)
- Plant height (Full Grown Plant) (cm)

Genotyping

- DNA Extraction
- PCR Analysis
- Gel Analysis (1.5% Gel)

Data Analysis

- Analyze our data by using Excel and Graphpad
- Used Box Plot
- One way ANOVA test


RESULTS

Result after Gel Electrophoresis

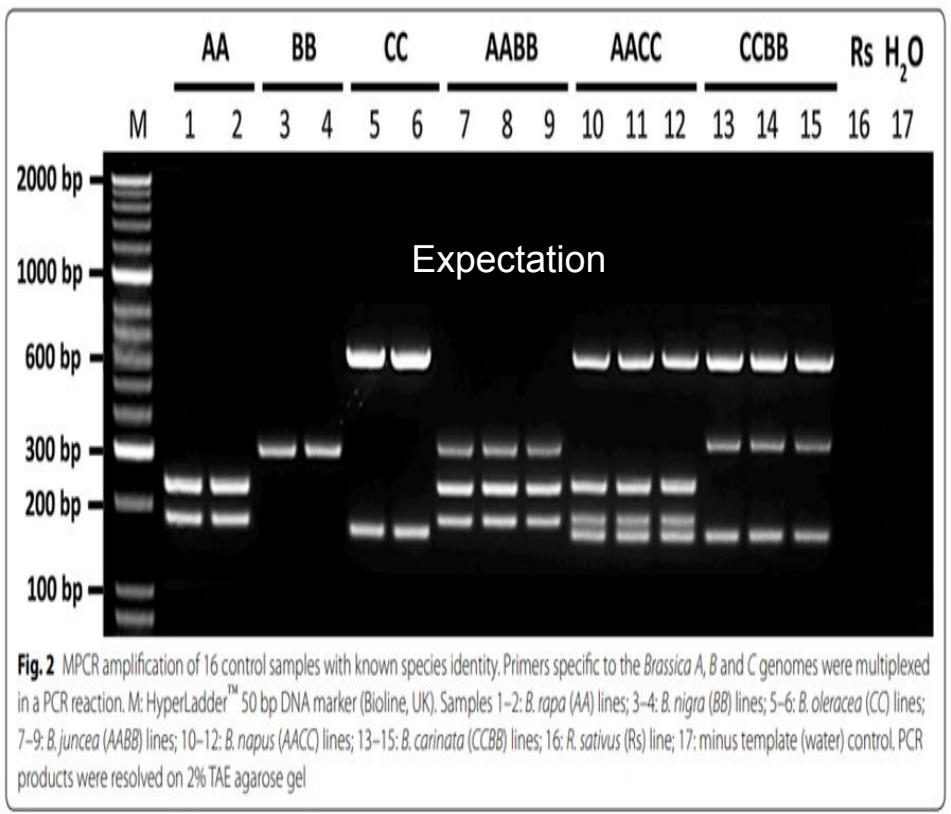
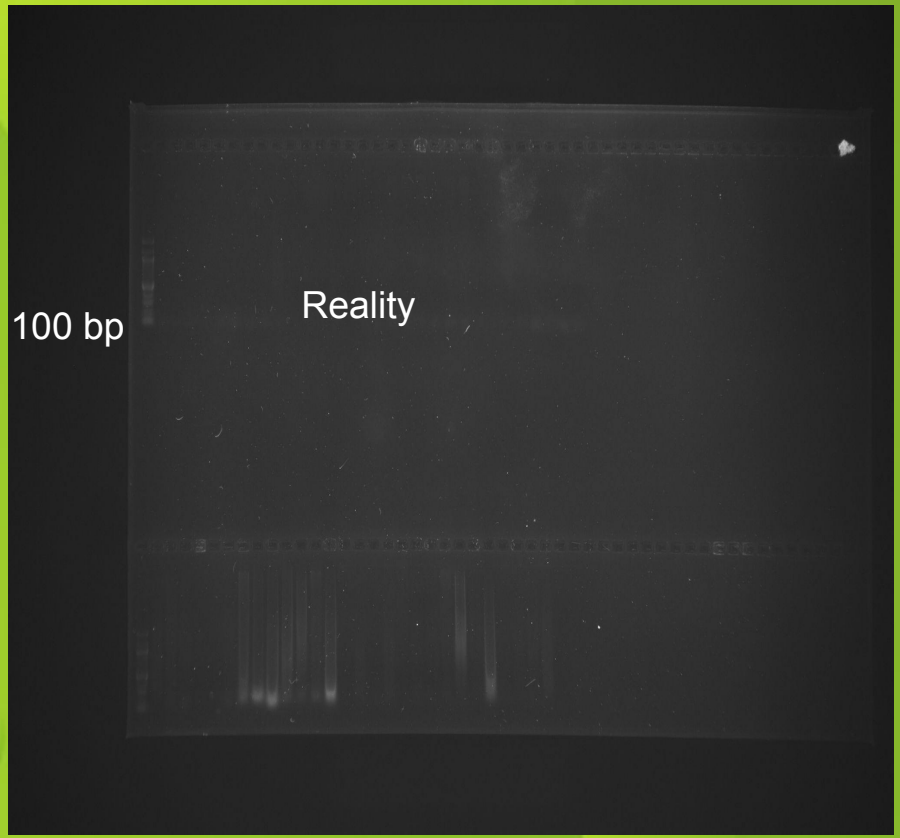


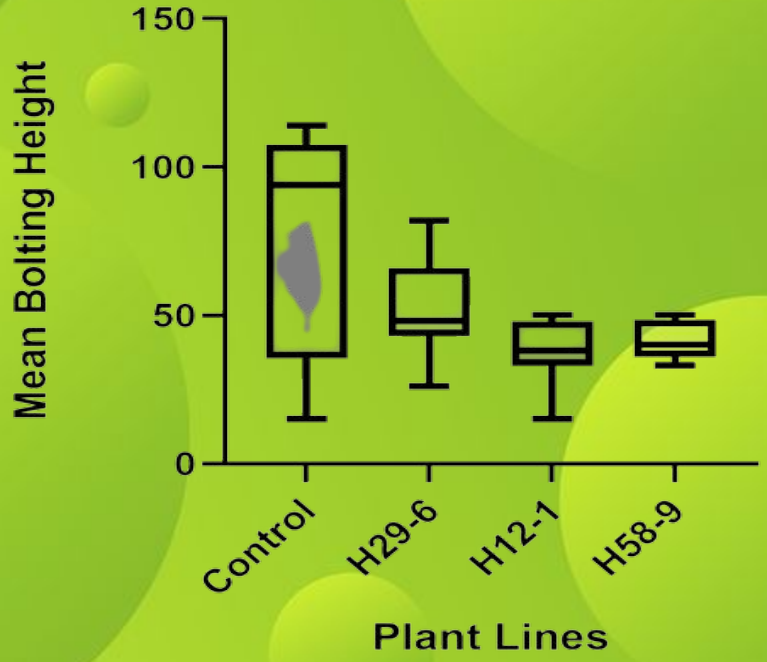
Fig. 2 MPCR amplification of 16 control samples with known species identity. Primers specific to the *Brassica A, B* and *C* genomes were multiplexed in a PCR reaction. M: HyperLadder™ 50 bp DNA marker (Bioline, UK). Samples 1–2: *B. rapa* (*AA*) lines; 3–4: *B. nigra* (*BB*) lines; 5–6: *B. oleracea* (*CC*) lines; 7–9: *B. juncea* (*AABB*) lines; 10–12: *B. napus* (*AACC*) lines; 13–15: *B. carinata* (*CCBB*) lines; 16: *R. sativus* (Rs) line; 17: minus template (water) control. PCR products were resolved on 2% TAE agarose gel



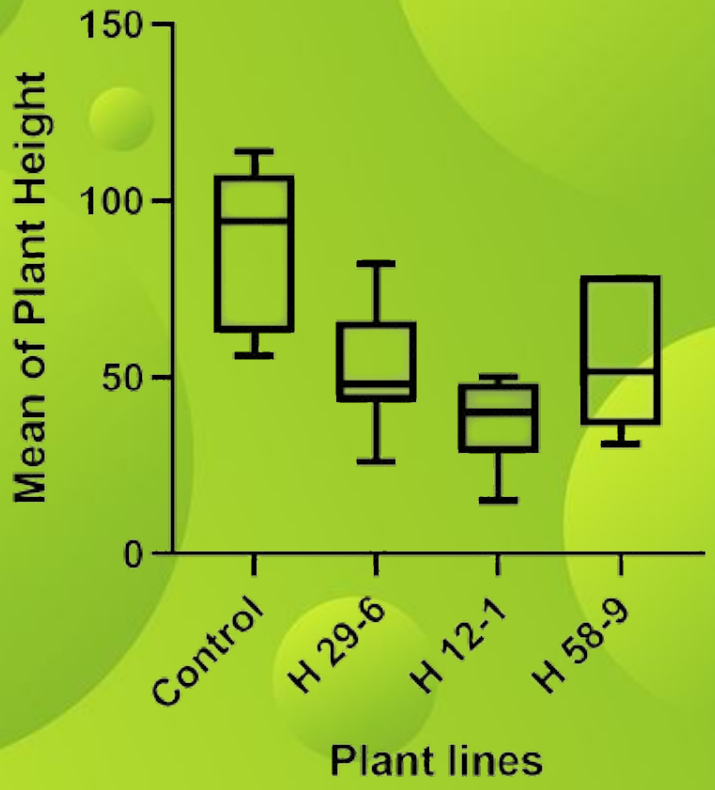
Printers are From Koh et al., 2017 A multiplex PCR for rapid identification of Brassica species in the triangle of U

Mean Rosette Diameter (Cm)
40
30
20
10
0
Control
H29-6
H12-1
H58-9
Plant line

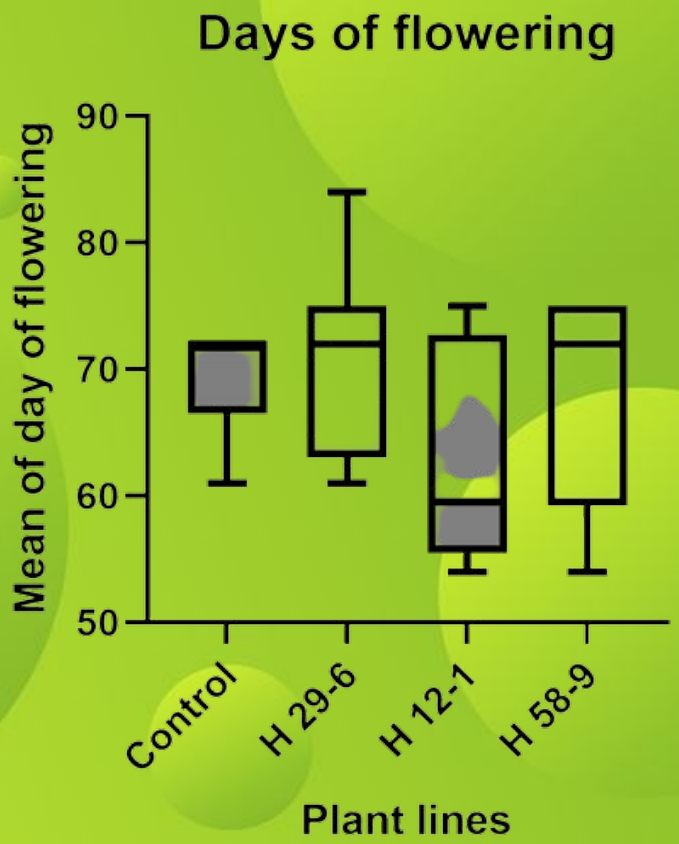
Bolting Height



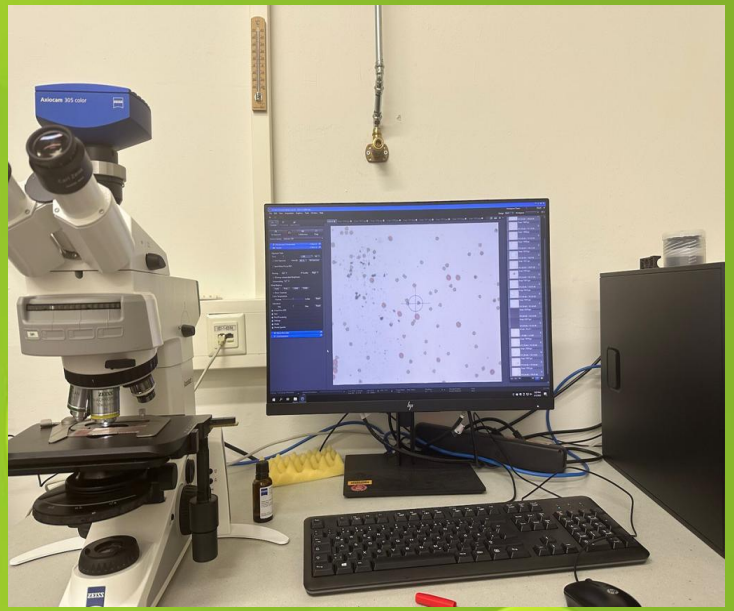
Note- Control includes Both J1 and C1 lineS


Plant Height (First Flowering)
Mean of Plant Height
150
100
50
0
Control
H 29-6
H 12-1
H 58-9
Plant lines

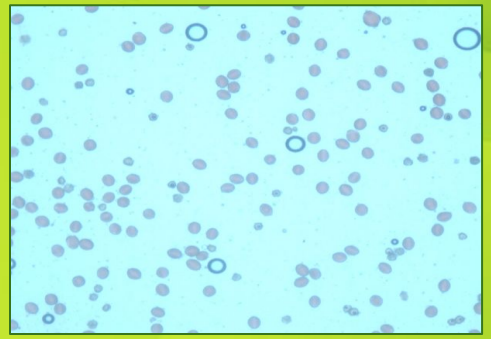

H12-1 - (S)


Days of flowering
Mean of day of flowering
90
80
70
60
50
Control
H 29-6
H 12-1
H 58-9
Plant lines

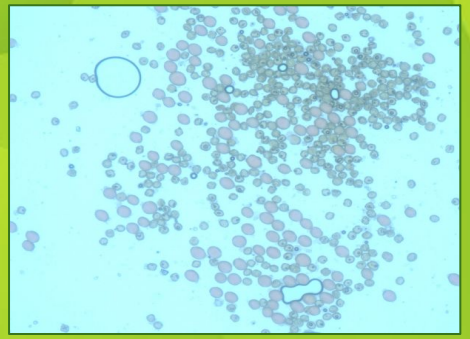
Pollen Viability



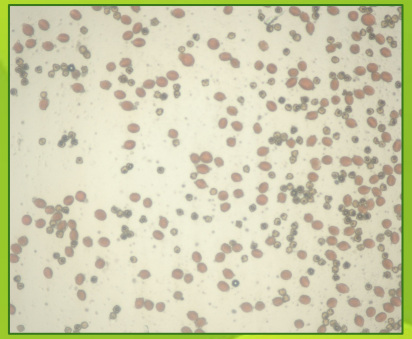
Pollen Viability



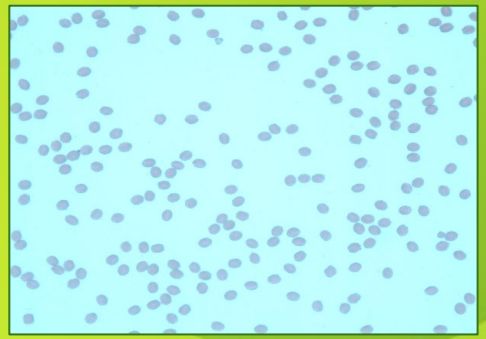
H 12-1-1



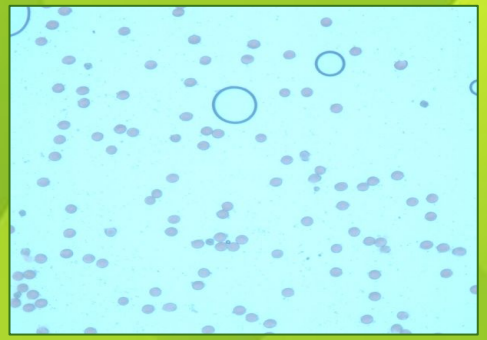
H 58-9-10



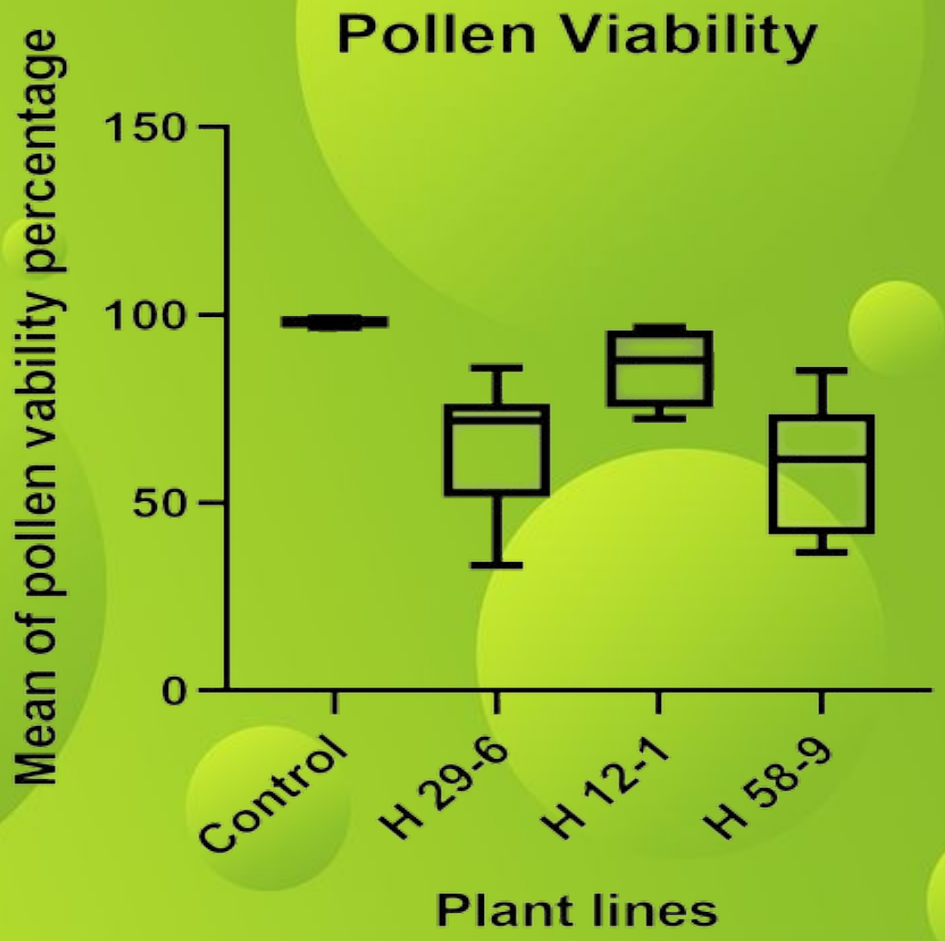
H 29-6-4



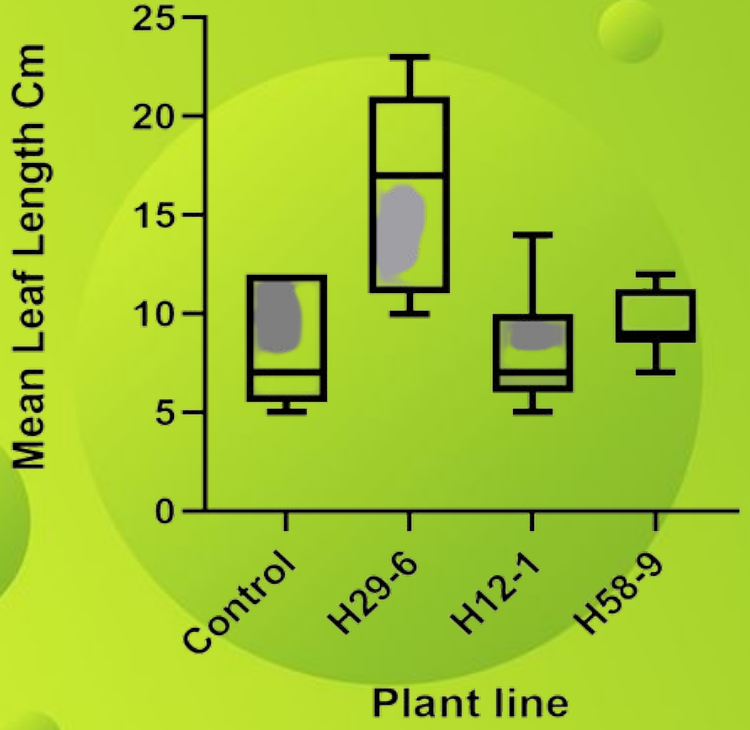
C1-1 (Control)

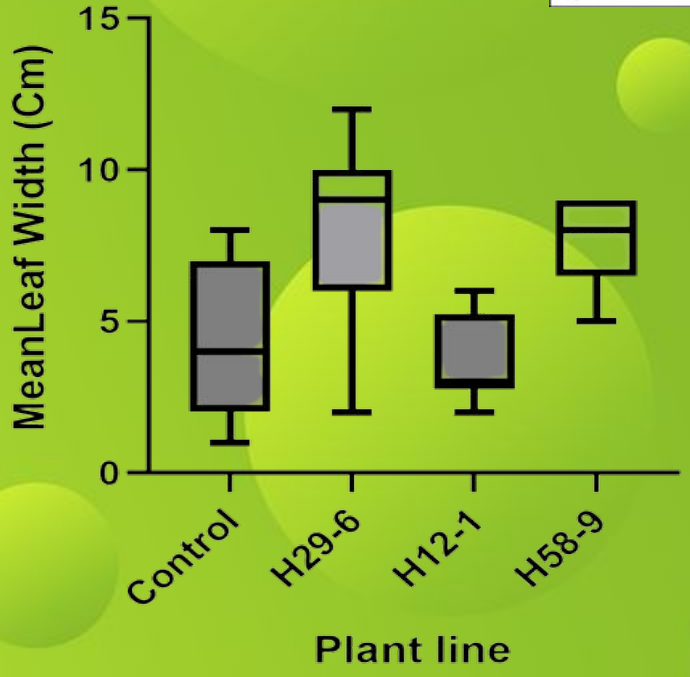


J2-1 (Control)


Pollen Viability
Mean of pollen vability percentage
150
100
50
0
Control
H 29-6
H 12-1
H 58-9
Plant lines

Leaf Dimensions Analysis

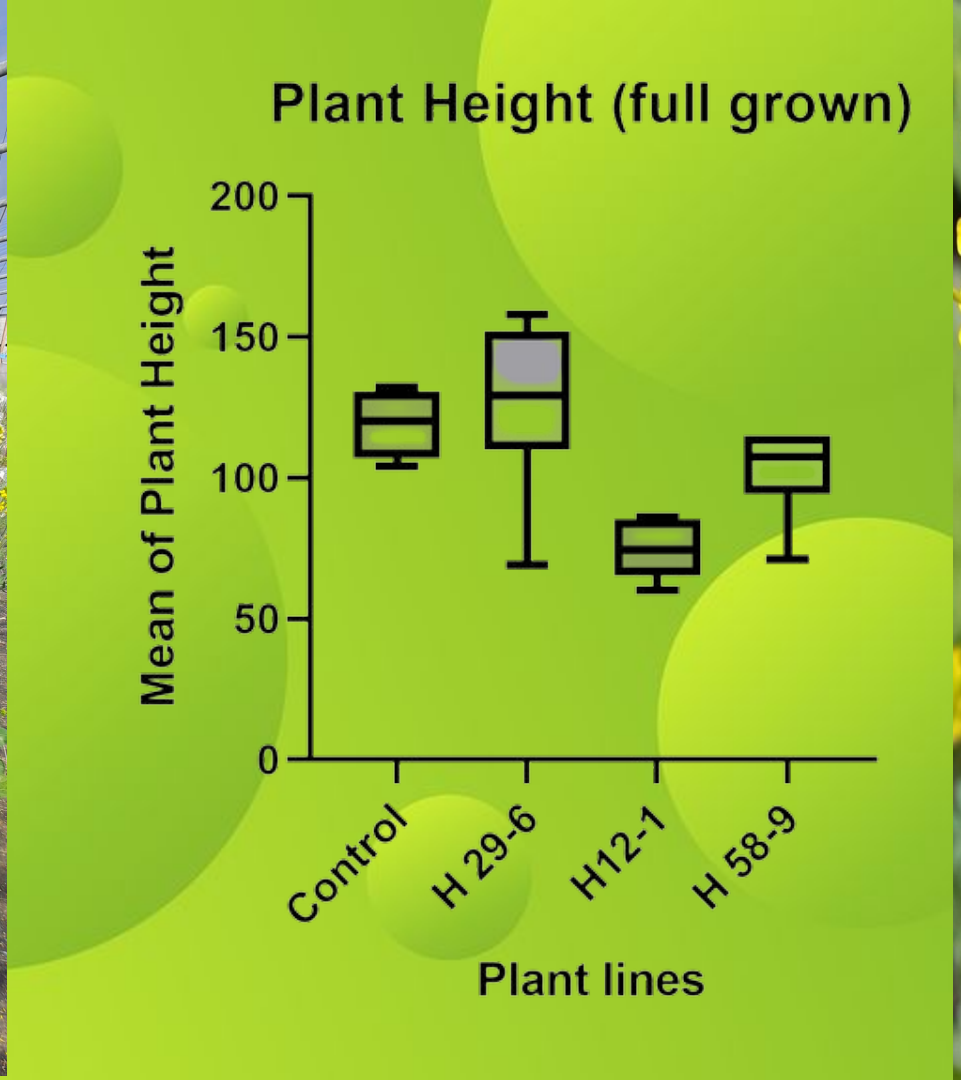





H22-1-(1)


H22-1-(2)


C-3


Plant Height (full grown)
Mean of Plant Height
200
150
100
50
0
Control
H 29-6
H12-1
H 58-9
Plant lines

WHAT'S THE TAKE HOME?

Conclusion

- Accordingly to our result, H 12-1 is the shortest rosette diameter, bolting height and plant height, earliest flowering, highest pollen viability and the shortest leaf length and width.
- H 12-1 line will be more tolerant under drought condition than H 29-6 and H 58-9.

Outlook

- Analyse short height and small leaf area Individuals
- Next Generation analysis to H12-1 line
- Validate this experiment
- Stabilising the autopolyploidy Brassica for yield and other Abiotic Stress.

Bollywood and Brassica
Thanks