



Plant Biotechnology

Bayer Russia Plant Biotechnology
Conference

July 2023





Soybean R0 and R0 Plant Production in Controlled Environments

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Soybean Production in Controlled Environments: Agenda

Topic 1

Overview of Soybean Production

Topic 2

Soybean Non-R0 Plants for Seed Harvest

Topic 3

Soybean R0 Plants for Seed Harvest

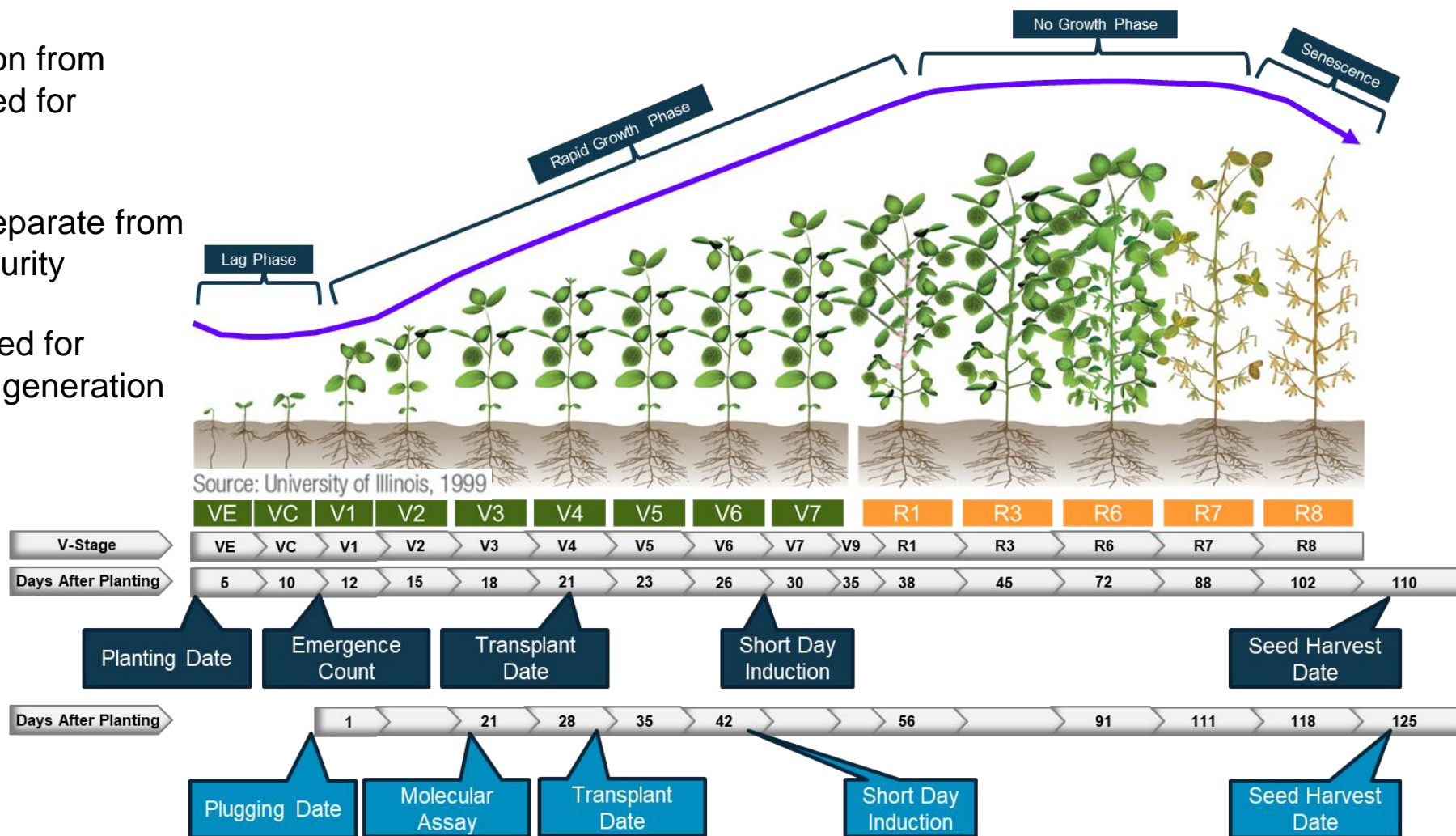
Topic 4

Summary



Overview of Soybean Production: Williams 82 Germplasm

- Non-R0 (Inbred) production from verified seed source if used for transformation
- Non-R0 must be grown separate from all other soy to maintain purity
- Non-R0 plants may be used for cotyledon harvest or next generation seed production
- R0 soy production from transformed plantlets that pass molecular screening
- R0 plantlets are developed more slowly than Non-R0 plants
- R0 population is more diverse than Non-R0 population





Environmental Control Parameters: Climate & Fertigation

- During rapid growth phase target even environment and increasing water/nutrients
- Plant growth slows with reproductive development
- As plant senescence (pod fill) occurs, target cooler and drier environment with less water/nutrients

Climate Controls	Vegetative	Reproductive	Range of Control Values
Average Daily Setpoint Temperature	Lower	Higher	26 – 28° C: higher temperatures at higher light levels
Day-Night Temperature Difference	Smaller	Larger	0 – 6° C
Start time for heating in the morning	Earlier	Later	4 hours before sunrise to sunrise
Afternoon temperature increase	None or small	Larger	0 – 2.5° C
Start Time for Day-Night Temperature Decrease	Earlier	Later	2 hours before to 2 hours after sunset
Speed of Day-Night Temperature Decrease	Slower	Faster	0 - 4° C per hour
Humidity deficit	Decrease	Increase	8 - 15 mB
Vapor Pressure Deficit	Lower	Higher	0.8 – 1.5 kPa
CO ₂ enrichment	More	Less	350 – 1500 ppm: Higher CO ₂ at higher light levels

Watering Controls	Vegetative	Reproductive	Range of Control Values
EC Growing Medium	Decrease	Increase	1.8 - 4.5 EC
EC Irrigation Water	Decrease	Increase	1.0 - 1.5 EC
Substrate Water Content	Increase	Decrease	45 - 65%
Day-Night Water Content Decrease	Decrease	Increase	2 - 10% (5 - 15% with Rockwool)
Irrigation cycle length and frequency	Short and Higher	Long and Lower	50 - 150 ml per dripper
Start time first irrigation	Earlier	Later	1 - 3 hours after sunrise/lights on
Stop time last irrigation	Later	Earlier	3 - 5 hours before sunset/lights off



Soybean Non-R0 Plant Production: Williams 82 Germplasm

Items of Special Interest

- All atypical plants should be discarded immediately
- Soybean are photoperiodic plants.
 - Reproductive transition driven by night lengths above 12 hours for Williams 82.
 - Facilities must be able to exclude all light during induction
 - Amount of vegetative growth before induction drives yield potential.
- Williams 82 germplasm target induction at V6-V8 to deliver > 300 seed per plant





Soybean Non-R0 Plant Production: Pre-Induction



Seed planted and
Germinated in
peat based media

28.3/24.4 C (83/76 F) D/N
16 hour photoperiod
Fertilizer upon emergence
with 15-5-15 at 1,000 μ S



Transplanting

V3-V4 Transplant soybean
21 DAP
Pot filled with peat based
media
Watered in with fertilizer
15-5-15 at 1,000 μ S



Production

29.4/22.8 C (85/73 F) D/N
16 hour photoperiod
Fertilizer 15-5-15 at 1,000 μ S
Wet/Dry cycles until well established roots
then even moisture
Induction at V6-V8



Soybean Non-R0 Plant Production: Pre-Induction

Suggested watering schedule with peat-based media

- 400 ml per minute with weighted emitters
- Wet/Dry cycles after transplant to promote root development
- Even moisture after well rooted

Time (DAP)	Approx. developmental stage	Approx. Irrigation Time	Days
5	VE	Sub-irrigation	Every Third Day (ETD)
10	VC	Sub-irrigation	Every Other Day (EOD)
12	V1.	Sub-irrigation	Every day (ED)
15	V2	Sub-irrigation	Every day (ED)
18	V3	Sub-irrigation	Every day (ED)
21	V4 - Transplant	Hand water at Transplant	As needed
23	V5	30 sec, one time (8 am)	Every day (ED)
25 - 27	V6 – Start short-day Induction – Reduce light to 10-12 hours once plants reach V6 – V8	45 sec, one time (8 am)	Every day (ED)
30	V7 - Start short-day Induction – Reduce light to 10-12 hours once plants reach V6 – V8	30 sec, two times (8 am, 4 pm)	Every day (ED)
32	V8 - Start short-day Induction – Reduce light to 10-12 hours once plants reach V6 – V8	45 sec, two times (8 am, 4 pm)	Every day (ED)



Soybean Non-R0 Plant Production: Post-Induction



Production

29.4/22.8 C (85/73 F) D/N
12 hour photoperiod
Fertilizer 15-5-15 at 1,000 μ S
Wet/Dry cycles until well
established roots then even
moisture
Induction at V6-V8



Dry Down

29.4/22.8 C (85/73 F) D/N
12 hour photoperiod
Fertilizer 15-5-15 at 1,000 μ S
R6 (Pod yellowing) reduce
water until 3-5% brown pods
then irrigation off



Harvest

29.4/22.8 C (85/73 F) D/N
12 hour photoperiod
5-10 days after irrigation off
Pods open when squeezed



Soybean Non-R0 Plant Production: Post-Induction

Suggested watering schedule with peat-based media

- 400 ml per minute with weighted emitters
- Even moisture after well rooted
- Lower water frequency as seed matures to promote drying

Time (DAP)	Approx. developmental stage	Approx. Irrigation Time	Days
32	V8 - Start short-day Induction – Reduce light to 10-12 hours once plants reach V6 – V8	45 sec, two times (8 am, 4 pm)	Every day (ED)
35	V9	30 sec, three times (8 am, 12 pm, 4 pm)	Every day (ED)
38	R1	45 sec, three times (8 am, 12 pm, 4 pm)	Every day (ED)
42	R2	30 sec, four times (7 am, 11 am, 2 pm, 6 pm)	Every day (ED)
45	R3	45 sec, four times (7 am, 11 am, 2 pm, 6 pm)	Every day (ED)
50	R4	45 sec, four times (7 am, 11 am, 2 pm, 6 pm)	Every day (ED)
55 – 58	R5	45 sec, four times (7 am, 11 am, 2 pm, 6 pm)	Every day (ED)
72	R6 – Begin to reduce irrigation	45 sec, three - four times (7 am, 11 am, 2 pm, 6 pm)	Every day (ED)
79	R 6.5	45 sec, three times (8 am, 12 pm, 4 pm)	Every day (ED)
87 - 90	R7	45 sec, two times (8 am, 4 pm)	Every day (ED)
93	R7	45 sec, one times (8 am)	Every day (ED)
95	R7 - Cut Stem, Stop Irrigation	None	Every day (ED)
100 – 105	R8 - Drying	None	Every day (ED)
107 -110	R8, Dry - Ready to Thresh	None	Every day (ED)



Soybean Non-R0 Plant Production: Mature Seed Path



Harvest

29.4/22.8 C (85/73 F) D/N
12 hour photoperiod
5-10 days after irrigation off
Pods open when squeezed



Threshing



Seed
Cleaning



Soybean Non-R0 Plant Summary: Williams 82 Germplasm

- R0 plants should be isolated from all other soybean plantings if seed will be used for cotyledon harvest or future transformation
- Environmental management early in crop cycle impacts later developmental stages
 - Atypical phenotypes should be removed as soon as detected
 - Williams 82 is sensitive to overwatering during lag and rapid growth phase
 - Williams 82 is sensitive to excessive salts in media. Avoid high EC levels.
- Soybeans are photoperiodic
 - > 12 hour photoperiod = vegetative for Williams 82
 - < 12 hour photoperiod = reproductive for Williams 82
- Short-day induction (10 to 12 hour photoperiod) is necessary for flowering
 - Vegetative growth before induction determines final seed yield
- Seed not used for cotyledon harvest may be advanced to next generation for future use.
 - Must maintain isolation throughout life cycle to ensure Williams 82 purity



Soybean R0 Plant Production: Williams 82 Germplasm

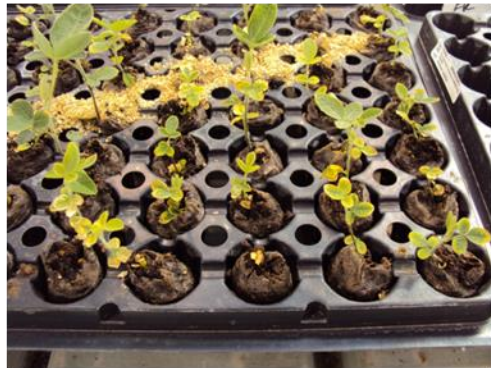
Items of Special Interest

- All atypical plants should be discarded immediately
- Soybean are photoperiodic plants.
 - Reproductive transition driven by night lengths above 12 hours for Williams 82.
 - Facilities must be able to exclude all light during induction
 - Amount of vegetative growth before induction drives yield potential.
- Williams 82 germplasm target induction for R0 plants at V6 to deliver ≈ 200 seed per plant





Soybean R0 Plant Production: Pre-Induction



R0 events in
peat-based plug



R0 Plug
Acclimation



Molecular
Selection

28.3/24.4 C (83/76 F) D/N
16 hour photoperiod
Mist + Domes + Shade for first 8 days after plugging
Fertilizer upon plugging with 15-5-15 at 1,000 μ S



Soybean R0 Plant Production: Pre-Induction

Suggested watering schedule with peat-based media

- Slow transition from tissue culture to greenhouse
- Day 1-8 mist, shade, and domes as needed especially in summer
- Sub-irrigation with 15-5-15 at 1,000 μ S
- Frequency varies by plant size and season

Time	Approx. developmental stage	Irrigation Type	Irrigation Time, Duration, & Frequency
Day 1-6	Plugging to V1	Sub-irrigation + Mist + Shade + Domes	Every Third Day (ETD), 15 sec. every 15 min, 30-40% shade, Dome
Day 7		Sub-irrigation + Mist + Shade	Every Other Day (EOD), 15 sec. every 15 min, 30-40% shade
Day 8		Sub-irrigation + Mist	Every Other Day (EOD), 15 sec. every 15 min
Day 9-13	V2-V3	Sub-irrigation	Every Other Day (EOD) or Every Day (ED) as needed
Day 14-21	V3	Sub-irrigation	1-2 x Every Day (ED)
Day 21-28	V3 – V5	Sub-irrigation	2-3 x Every Day (ED)
Day 28 +	V4+	Drippers	Up to 4 x Daily



Soybean R0 Plant Production: Pre-Induction Acclimation



Molecular Selection

28.3/24.4 C (83/76 F) D/N
16 hour photoperiod
Fertilizer 15-5-15 at 1,000 μ S



Transplanting

V3-V5 Transplant soybean
 \approx 28 days after plugging
Pot filled with peat based media
Watered in with fertilizer 15-5-15
at 1,000 μ S



Production

29.4/22.8 C (85/73 F) D/N
16 hour photoperiod
Fertilizer 15-5-15 at 1,000 μ S
Wet/Dry cycles early then even
moisture
Induction at V6



Soybean R0 Plant Production: Pre-Induction Production

Suggested watering schedule with peat-based media

- 400 ml per minute with weighted emitters
- Wet/Dry cycles after transplant to promote root development
- Even moisture after well rooted

Time (DAP)	Approx. developmental stage	Approx. Irrigation Time	Days
28	V4 +	Hand Irrigation at Transplant	Every day (ED)
35 - 42		20-25 sec, one time (8 am)	Every Other Day (EOD)
43 - 49	V6 – Reduce light to 12 hours per day. Induction started.	30 sec, one time (8 am)	Every day (ED)
50 - 56		30 sec, two times (8 am, 4 pm)	Every day (ED)



Soybean R0 Plant Production: Post-Induction



Production

29.4/22.8 C (85/73 F) D/N
16 hour photoperiod
Fertilizer 15-5-15 at 1,000 μ S
Wet/Dry cycles early then even
moisture
Induction at V6



Production

29.4/22.8 C (85/73 F) D/N
12 hour photoperiod at V6
Fertilizer 15-5-15 at 1,000 μ S
Even moisture then decrease
Trim excessive growth



Dry Down

29.4/22.8 C (85/73 F) D/N
12 hour photoperiod
Fertilizer 15-5-15 at 1,000 μ S
R6 (Pod yellowing) reduce
water until 3-5% brown pods
then irrigation off



Harvest

29.4/22.8 C (85/73 F) D/N
12 hour photoperiod
5-10 days after irrigation off
Pods open when squeezed



Soybean R0 Plant Production: Post-Induction Production

Suggested watering schedule with peat-based media

- 400 ml per minute with weighted emitters
- Wet/Dry cycles after transplant to promote root development
- Even moisture after well rooted

Time (DAP)	Approx. developmental stage	Approx. Irrigation Time	Days
43 - 49	V6 – Reduce light to 12 hours per day. Induction started.	30 sec, one time (8 am)	Every day (ED)
50 - 56		30 sec, two times (8 am, 4 pm)	Every day (ED)
55 - 63	R1	45 sec, two times (8 am, 4 pm)	Every day (ED)
64 - 70		30 sec, three times (8 am, 12, pm, 4 pm)	Every day (ED)
71 - 77		30 sec, three times (8 am, 12, pm, 4 pm)	Every day (ED)
78 - 84		30 sec, three times (8 am, 12, pm, 4 pm)	Every day (ED)
85 - 91		30 sec, three times (8 am, 12, pm, 4 pm)	Every day (ED)
92 - 98	R5 to R6 - Begin decreasing water.	30 sec, three times (8 am, 12, pm, 4 pm)	Every day (ED)
99 - 106		30 sec, two times (8 am, 4 pm)	Every day (ED)
107 - 110		30 sec, one time (8 am)	Every day (ED)
111 - 117	R8 - Cut Stem, Stop Irrigation	30 sec, one time (8 am) then None	Every day (ED)
118 - 124	R8, Dry - Ready to Thresh	None	Every day (ED)

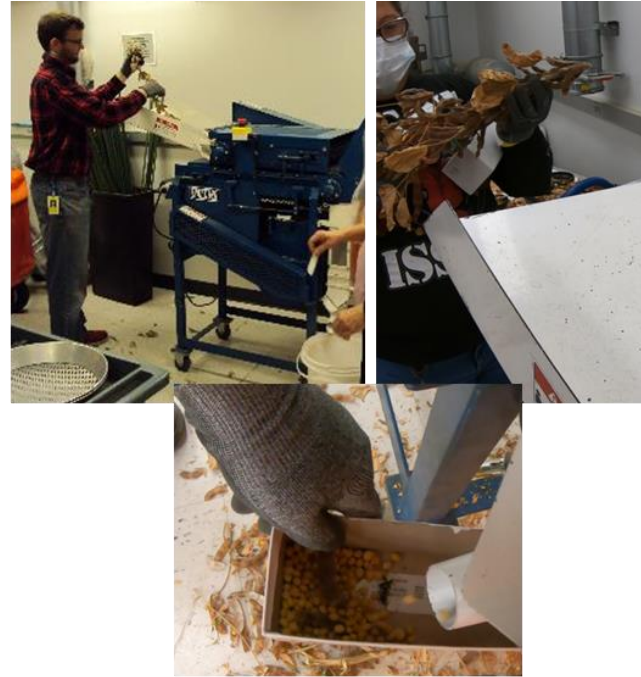


Soybean R0 Plant Production: Mature Seed Path



Harvest

29.4/22.8 C (85/73 F) D/N
12 hour photoperiod
5-10 days after irrigation off
Pods open when squeezed



Threshing



Seed
Cleaning



Soybean R0 Plant Summary: Williams 82 Germplasm

- R0 plants should be isolated from all other soybean plantings
- Environmental management early in crop cycle impacts later developmental stages
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 - > 12 hour photoperiod = vegetative for Williams 82
 - < 12 hour photoperiod = reproductive for Williams 82
- Short-day induction (10 to 12 hour photoperiod) is necessary for flowering
 - Vegetative growth before induction determines final seed yield



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Thank you!



Any questions?

