Using Plant Biotechnology for Alfalfa Improvement

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Topics for Discussion

- Use of Plant Biotechnology to Modify Traits that Influence Crop Production

- Production of “Transgenic” (Genetically Engineered) Alfalfa

Trait Modification Using Biotechnology

1. Factors Effecting Crop Production
   a. Abiotic Stress
   b. Pathogens and Pests

2. Quality or “value added” Characteristics
   a. Enhanced shelf life
   b. Modified nutritional profiles

Transgenic Plants with Tolerance to Abiotic Stress

1. Cold Stress
   a. modification of chloroplast membranes (plant, algae)
   b. expression of fish antifreeze proteins
   c. reduced production of free-radicals (plant)

Transgenic Plants with Tolerance to Abiotic Stress

2. Drought and Salinity
   a. production of osmoprotectants
      - fructans (bacteria)
      - mannitol (bacteria)
      - glycine betaine (plant)
   b. reduced production of free radicals (plant)

Transgenic Plants with Resistance to Pathogens and Pests

2. Viruses
   a. expression of normal/modified viral genes
      coat protein, replicase, movement protein
   b. commercial products: Newleaf Plus ^{TM} Potato ($PLRV^{R}$)
      Squash ($ZYMV^{R}$)

Transgenic Plants with Resistance to Pathogens and Pests

3. Fungi
   a. Expression of plant genes that are activated in response to pathogen attack.

      Phytoalexins, chitinases, glucanases

   b. Resistance levels are not adequate for commercialization

Transgenic Plants with Tolerance to Abiotic Stress

3. Trace Metal Contamination of Soil

   a. aluminum (bacteria)

   b. mercury (bacteria)

   c. cadmium (mouse)

Transgenic Plants with Resistance to Pathogens and Pests

1. Insects
   a. delta-endotoxin gene (*B. thuringiensis*)
      commercial products: Bollgard ™ Cotton
      Newleaf ™ Potato

   no effective Bt genes against lygus, mites, thrips

   b. Protease inhibitor genes (plants)
   c. Vegetative insecticidal proteins (bacteria)

Transgenic Plants with Resistance to Pathogens and Pests

4. Bacteria
   a. Expression of bacterial genes that confer resistance to bacterial toxins

      P. syringae pv. phaseolicola - OCTase (phaseolotoxin ^{R})

      P. syringae pr. tabaci - Acyltransferase (tabtoxin^{R})

   b. Expression of antimicrobial genes of diverse origin

      insects, mammals, plants, bacteriophage

Expression of T4 Lysozyme Gene in Potato and effect on Resistance to *Erwinia caratovora*

Genotpe	Tuber rot (%) after inoculation with different # bacteria/tuber			
	1000	5000	40000	125000
Wildtype	75	90	90	100
Transgenic control	60	85	85	90
T4 transgenic	5	30	45	65

[1]During et al. 1993. Plant J. 3: 587-598

Transgenic Alfalfa

Gene	Origin	Purpose
NPT 2 (Kanamycin^r)	bacteria	selection
Pat (phosphorothricine^r)	bacteria	selection
Hpt (hygromycin^r)	bacteria	selection
GUS (B-glucuronidase)	bacteria	selection/expression
LUX (luciferase)	coral, bacteria	selection/expression
Bar	bacteria, synthetic	Roundup Ready
B-phaseolin	plant	protein accumulation

Transgenic Alfalfa

Gene	Origin	Purpose
Alfalfa lectin	plant	Alfalfa-*Rhizobium*
Sunflower albumin	plant	nutritional quality
AMV coat protein	virus	virus resistance
glucanase	plant	fungal resistance
delta-endotoxin	bacteria	insect resistance
chitinase	bacteria	insect resistance
Superoxide dismutase	plant	drought tolerance freezing tolerance

Insect Resistance in Transgenic Alfalfa

Nicolai Strizov et al 1996 PNAS 93: 15012 - 15017

Objective: Express bacterial chitinase and delta-endotoxin genes in alfalfa and evaluate insect resistance in transgenic plants.

Materials and Methods:

1. Transformed *M. sativa* var. Regen S clone RA3

2. pN56 = delta - endotoxin
   pN57 = delta-endotoxin + chitinase

3. Evaluate against
   a. *Spodoptera littoralis* (cotton leafworm)
   b. *S. exigua* (beet armyworm)

Control of Cotton Leafworm (*S. littoralis*)

	Larval Mortality		
Construct	95-100%	30-90%	<30%
WS6	55.5%	18.5%	33.3%
WS7	43.8%	15.6%	40.6%

Control of Beet Armyworm (*S. exigua*)

	Larval Mortality (%)	
Instar	WS6	WS7
1	100(1)	100(3)
2-3	100(5)	100(7)
3-4	100(2)	100(3)
4-6	100(3)	100(2)

Transgenic Alfalfa with Resistance to Abiotic Stress

Bryan McKersie et al. 1993. Plant Physiol. 103:1155-1163

1996. Plant Physiol. 111:1177-1181

Objective: Express a superoxide dismutase (SOD) gene in transgenic alfalfa and evaluate plants for tolerance to drought and freezing.

Why Superoxide Dismutase?

1. Activated oxygen (0_2^-) levels are enhanced by exposure to environmental stress.
2. Activated oxygen can produce hydroxyl radicals (OH^-)
3. Hydroxyl radicals cause mutations and damage proteins and cell membranes.
4. Tolerance to abiotic stress in plants is associated with the ability detoxify activated oxygen.
5. SOD detoxifies activated oxygen.

Shoot Regrowth (%) After Drought Stress

Days of Drought Stress

Plant	1	3	5	7	9
RA3	85	100	90	75	25
SOD-5	100	100	95	110	85
SOD-30	135	115	105	80	20

Herbage Yield (g dry matter m-^{2}) in Field

Plant	1992	1993	1994
RA3	151	265	37
SOD-30	291	465	140
SOD-64	286	652	198
SOD-5	189	353	135
SOD-38	266	510	193
A7	342	956	860
A9	321	920	848
LSD (0.05)	130	150	104

Materials and Methods

a. Transformed *M. sativa* Var. Regen S clone RA3

b. Evaluated plants for:

shoot regrowth after freezing
shoot regrowth after drought stress
herbage yield in field trials
survival in the field

Results

Shoot regrowth (mg dry matter/plant) after freezing

at -8 to 1 16°c for 48 hours

Plant	No Stress	Freezing
RA3	229	46
SOD-5	202	73
SOD-38	181	54
SOD-30	220	108
SOD-64	182	75

Survival of Plants in Field

Plant	Fall 92	Fall 93	Fall 94
RA3	97	42	17
SOD-30	88	27	26
SOD-64	98	67	57
SOD-5	88	57	42
SOD-38	94	66	66
A7	100	89	88
A9	94	83	77
LSD (0.05)	NS	22	15

Challenges

1. Tetraploid genome: requires dominant genes
2. Variation between plants in variety
3. Difficulty in transformation of commercial varieties
4. Proprietary restrictions on the use of technology