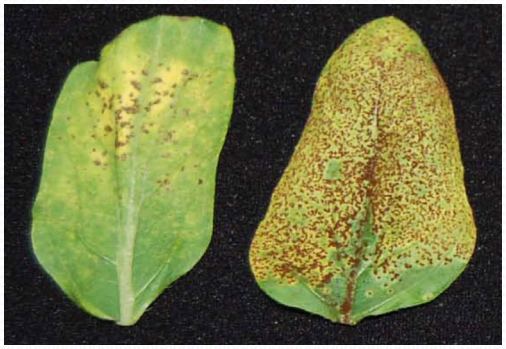
2021 Progress for enhancing rust resistance in confection sunflower production through next-generation technologies

Guojia Ma[1], Xuehui Li[1], Lili Qi[2]

[1]NDSU, Dept. of Plant Sciences
[2]USDA-ARS, Northern Crop Science Laboratory

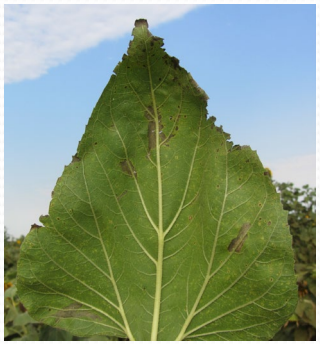
Main causal organism: fungus *Puccinia helianthi*

Greenhouse Screening



R S

Field Screening



R S

Rust threats sunflower production

- One of the most serious diseases of sunflower in the world with an increasing importance in North America in recent years
- Number of North American (NA) rust races identified currently: 38
- Control: Registered fungicides and host resistance
- Using of resistant hybrids is most effective management tool (economic & environmental)

Genetics of rust resistance in sunflower

- Single dominant genes in sunflower control rust resistance
- A total of 17 rust resistance (R) genes have been discovered in sunflower
- New rust races make current R genes ineffective
  - Out of 17 rust R genes, 7 (R_{11}, R_{12}, R_{13a}, R_{13b}, and R_{14}-R_{16}) remain effective to all rust races

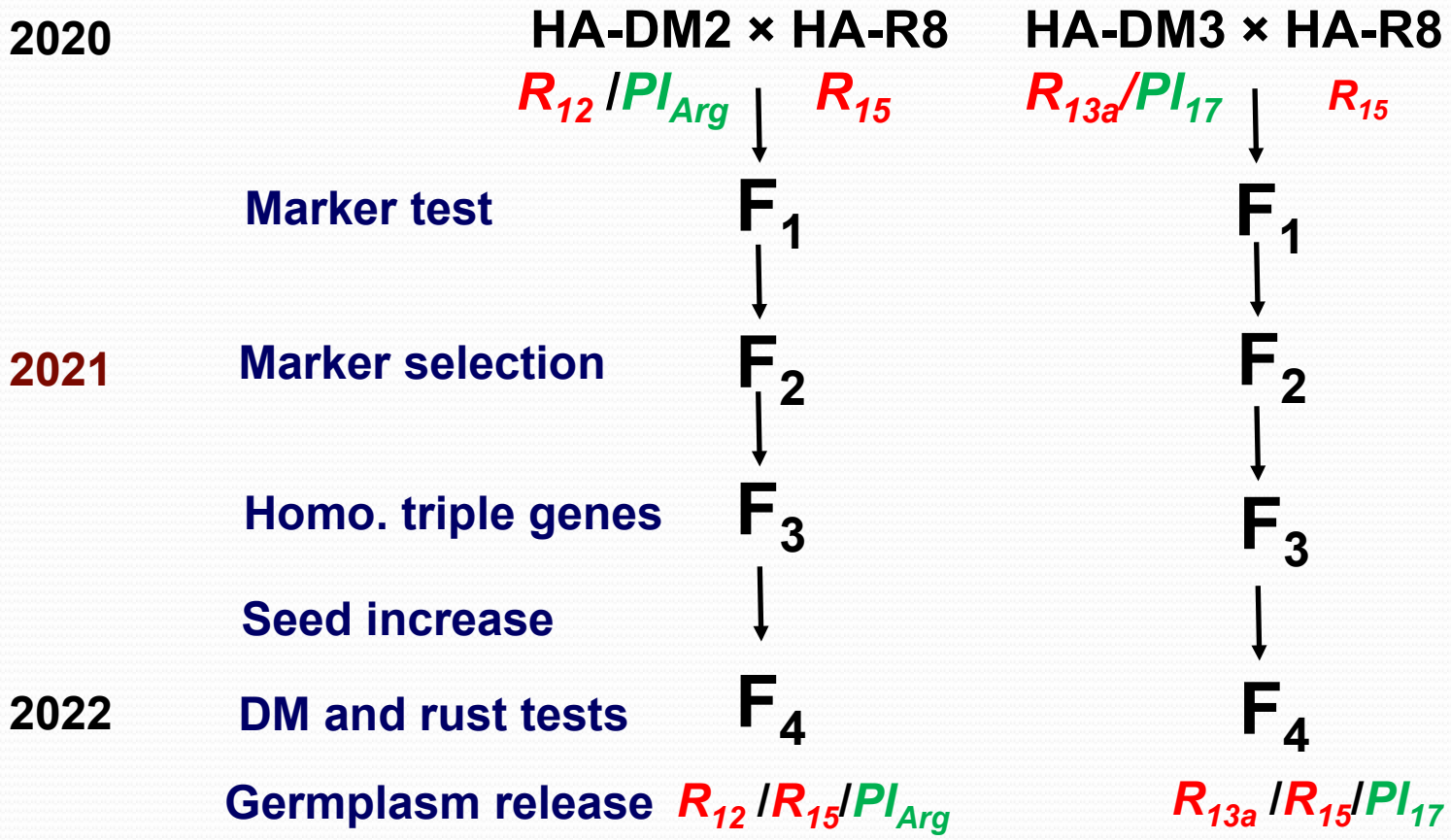
Research objectives (2020-2022)

- Stack effective rust *R* genes to generate multi-disease resistant lines
- Construct high density SNP genetic maps of R_{13a}, R_{12}, and R_{15}
- Develop diagnostic molecular markers for three genes
- Identify candidate genes associated with rust resistance in the sunflower genome

Research objectives (2021)

- Continue to stack effective rust *R* genes to generate lines with improved rust resistance
- Complete fine mapping of R_{13a}
- Develop diagnostic markers for R_{13a}
- Identify candidate genes of R_{13a}
- Saturation mapping of R_{12}

R genes stack



Fine mapping of R_{13a}

- 2020: whole genome sequence of HA-R6 (R_{13a}); tested 432 SNP markers; mapped 7 SNPs to the gene target region, which co-segregated with R_{13a}

- 2021: fine mapping of R_{13a}
  - Two markers were used to screen recombinants from 2,820 individuals of a large population
  - Identified 312 recombinants
  - Evaluated rust resistance of R_{13a} recombinant families
  - Genotyped R_{13a} recombinants

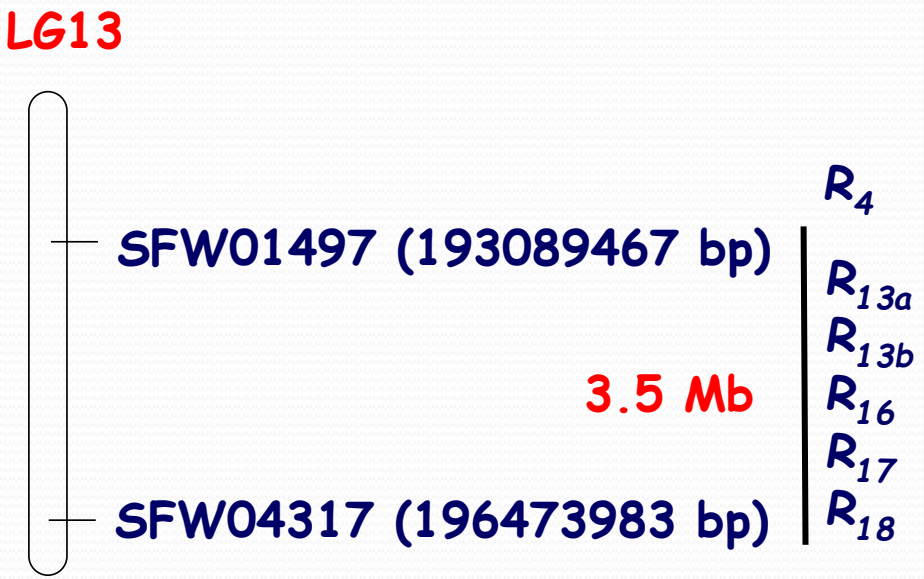
Table 1 Summary of R_{13a} fine mapping results

Marker	No. recombination	Genetic distance (cM)	Physical position on XRQr1.0 assembly (bp)
SFW01497	0	0	193089467-193089349
C13_194268343	30	0.5319	194268143-194268543
C13_194735854	5	0.0887	194735654-194736054
C13_194757055	4	0.0709	194756855-194757255
R_{13a}	7	0.1241	-
C13_195501970	20	0.3546	195501770-195502170
C13_195522913	0	0.0000	195522713-195523113
C13_195526945	0	0.0000	195526745-195527145
C13_195556768	0	0.0000	195556568-195556968
SFW04275	21	0.3723	196464687-196464768
SFW04317	1	0.0177	196474077-196473983
SFW05743	4	0.0709	196521145-196521026
HT382	106	1.8794	-

Reducing R_{13a} interval from 3.5 Mb to 0.745 Mb

Fine mapping of R_{16}

- R_{16} in TX16R germplasm line and mapped to the same region as R_{13a}



- **Saturation mapping of R_{16}**

  - TX16R (R_{16}) was sequenced at 40x genome coverage
  - Selected a total of 432 SNPs from TX16R (R_{16}) and HA-R6 (R_{13a}) whole genome sequences
  - Screened the parents of HA 434 and TX16R
  - Mapped 16 SNPs to the R_{16} target region

- **Fine mapping of R_{16}**
  - Two flanking markers were used to screen recombinants from 2,256 individuals of a large population
  - Identified 203 recombinants
  - Evaluated rust resistance of the R_{16} recombinant families
  - Genotyped the R_{16} recombinants

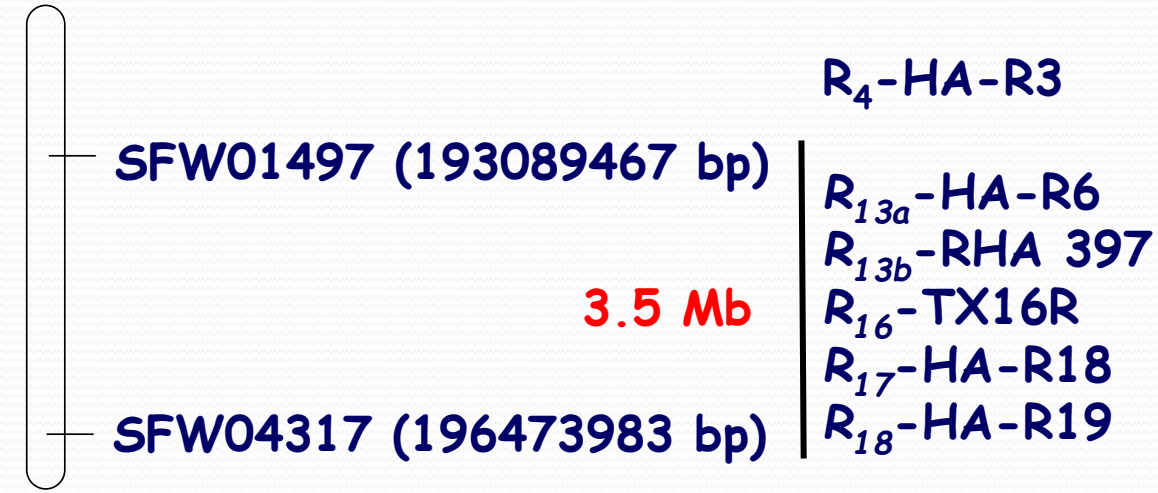
Table 2 Summary of R_{16} fine mapping results

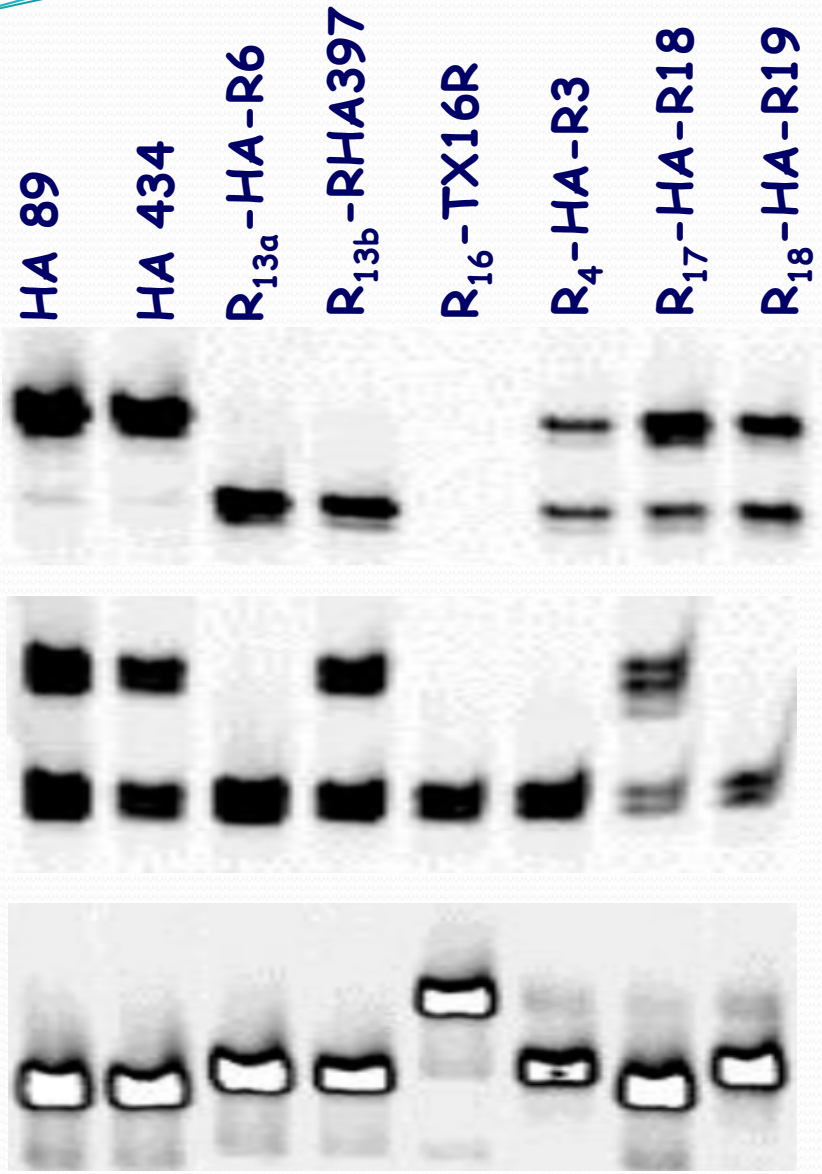
Marker	No. recombination	Genetic distance (cM)	Physical position on XRQr1.0 assembly (bp)
ORS316	0	0	-
SFW8875	110	2.4379	193131235-193131123
C13_194722668	43	0.9530	194722468-194722868
R_{16}	8	0.1773	-
C13_195512786	6	0.1330	195512586-195512986
C13_195552917	0	0.0000	195552717-195553117
C13_195605372	0	0.0000	195605172-195605572
C13_195836770	7	0.1551	195836570-195836970
C13_195840634	0	0.0000	195840434-195840834
C13_195874138	0	0.0000	195873938-195874338
SFW05743	31	0.6871	196521145-196521026

Reducing R_{16} interval from 3.5 Mb to 0.79 Mb

Marker specificity test of R_{13a} and R_{16}

A total of 16 SNP markers mapped to 3.5 Mb region were selected to test eight lines, including two susceptible lines, HA 89 and HA 434, and six resistant lines



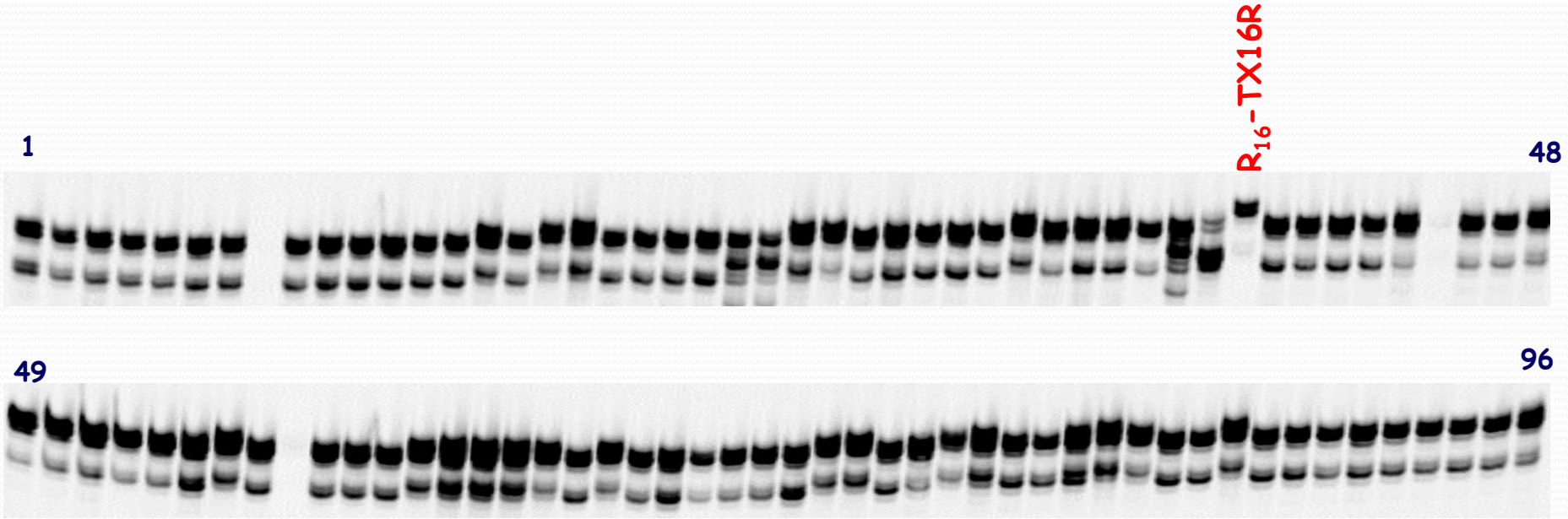


C13_19428343: R_{13a} shares the marker allele with R_{13b}

C13_195501970: R_{13a} shares the marker allele with R_{16}, R_4, and R_{18}

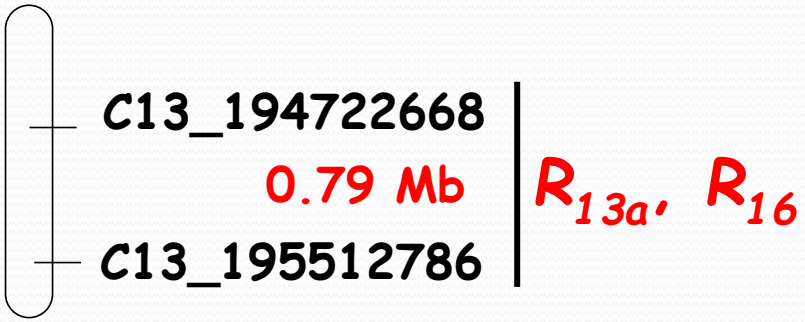
C13_195874538: distinguishing R_{16} from all genes in the cluster

Ten SNP markers were selected to test 96 sunflower lines, and only C13_194722668 can distinguish R_{16} from all other lines



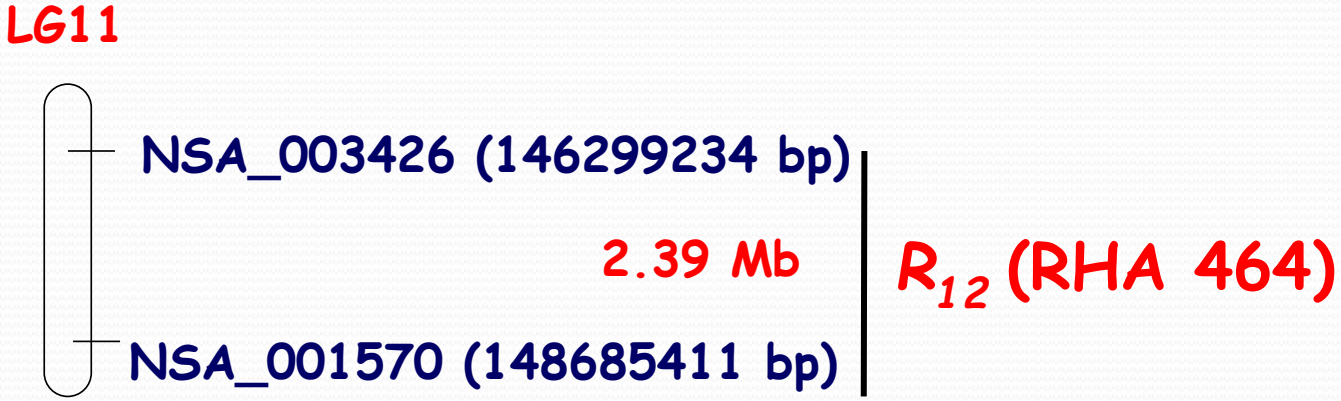
Candidate genes for R_{13a} and R_{16}

LG13



Gene name	Physical position (bp)		Lentgh (bp)	Description
	Start	End		
HanXRQChr13g0425851	194725998	194753531	27534	Putative NB-ARC
HanXRQChr13g0425891	194800201	194803684	3484	Putative NB-ARC
HanXRQChr13g0425931	195196820	195210745	13926	Putative NB-ARC
HanXRQChr13g0425941	195250038	195252703	2666	Putative NB-ARC

Saturation mapping of R_{12}



- **SSR marker development**

  - Extracted 3.5 Mb sequence covering R_{12} from the reference genome of HA412-HO
  - Designed 58 pairs of SSR primers from the sequence
  - Screened the polymorphism between HA 89/RHA 464 (R_{12})
  - Genotyped 140 F_2 individuals with 6 polymorphic markers

SNP marker development

- Sequenced RHA 464 (R_{12}) with 40x genome coverage
- Called SNPs and InDels from the XRQ and HA412-HO genome sequences.
- Selected 186 SNPs from the gene target region
- Screened the polymorphism between HA 89/RHA 464 (R_{12})
- Genotyped 140 F_2 individuals with 52 polymorphic markers
- Finally, 4 SSR and 44 SNP markers were mapped to the R_{12} target region.

Future work (2022)

- Complete fine mapping of R_{12}
- Test and validate diagnostic markers for R_{12}
- Identify candidate genes of R_{12}
- Prepare manuscript
- Saturation and fine mapping of R_{15}
- Release the germplasms resistant to rust and downy mildew

Acknowledgements

- Angelia Hogness (USDA-ARS, NCSL)
- Dr. Zahirul Talukder (NDSU/USDA-ARS, NCSL)
- Dr. Md Shamimuzzaman (USDA-ARS, NCSL)
- Dr. Qijian Song (USDA-ARS, BARC)

Financial Support

- National Sunflower Association
- Specialty Crop Block Grant, USDA-AMS
- ND Department of Agriculture

USDA U.S. DEPARTMENT OF AGRICULTURE AMS AGRICULTURAL MARKETING SERVICE

National Sunflower ASSOCIATION

NORTH DAKOTA DEPARTMENT of AGRICULTURE

NDSU NORTH DAKOTA STATE UNIVERSITY


Thank you