

# A new male-sterile *Helianthus tuberosus* cytoplasm and its fertility restoration gene providing alternatives for hybrid sunflower production

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# Introduction

- For over 40 years, the hybrid sunflower seed industry has largely relied on **CMS PET-1** and its corresponding *Rf<sub>1</sub>* gene
- **Alternative CMS/*Rf* systems** could expand the diversity of the sunflower crop, and reduce the risks inherent with using a single CMS/*Rf* system
- **72** sunflower CMS sources have been identified, but only about a **half** of them have known corresponding *Rf* genes
- Only **7** *Rf* genes have been mapped

# The *Rf* genes and their position on linkage group

Gene	CMS type	Linkage group (LG)
<i>Rf</i> <sub>1</sub> , <i>Rf</i> <sub>5</sub>	CMS PET-1	13
<i>Msc</i> <sub>1</sub>	CMS PET-1	12 of the RFLP map of Gentsbittel et al. (1999)
<i>Rf</i> <sub>3</sub> -RHA 340, <i>Rf</i> <sub>3</sub> -RHA 280	CMS PET-1	7
<i>Rf</i> <sub>4</sub>	CMS GIG2	3
<i>Rf</i> -PEF1	CMS PEF1	an AFLP LG that differed from 13

# Introduction (cont.)

- ◉ **Amphiploids**, derived from chromosomally doubled interspecific or intergeneric crosses, have been used as an **important “bridge”** for transferring different genes
- ◉ Recently, **CMS 514A**, derived from the cross between *H. tuberosus* and an inbred line **7718B**, was developed at the Liaoning Academy of Agricultural Sciences, Liaoning, China

## No restorer for CMS 514A was found with 33 maintainer and restorer lines

Material Source	Number	To CMS PET-1	To CMS 514A
Italy	6	R	B
Italy	5	B	B
China	13	R	B
China	2	B	B
U.S.A	4	R	B
U.S.A	1	B	B
France	1	R	B
Australia	1	R	B

# No restorer for CMS 514A was found with the 20 tester lines from USDA-ARS-NCSL

Material	Plant No.	Sterile No.	Material	Plant No.	Sterile No.
514A / HA 821	44	44	514A / RHA 294	21	21
514A / HA 801	21	21	514A / Armavir	39	39
514A / Seneca	18	18	514A / VNIIMK	37	37
514A / Hopi Dye	30	30	514A / P21	32	32
514A / Smena	38	38	514A / RCMG3	46	46
514A / Luch	37	37	514A / RHA 290	47	47
514A / Issanka	42	42	514A / HA 89	36	36
514A / RHA 266	42	42	514A / RCMG1	39	39
514A / RHA 276	36	36	514A / RCMG2	47	47
514A / RHA 274	32	32	514A / Peredovik	46	46

# Objectives

- ◉ Identify the *Rf* gene for CMS 514A
- ◉ **Introgress** the *Rf* gene into a cultivated sunflower background and study the **inheritance** of the *Rf* gene
- ◉ Characterize the **alien chromosome or segments** in the progenies
- ◉ **Map** the *Rf* gene using SSR and EST-SSR markers

# Materials and Methods

- Five interspecific amphiploids ( $2n=68$ )
  - Amp *H. atrorubens*/HA 89
  - Amp *H. mollis*/P 21
  - Amp *H. cusickii*/P 21
  - Amp *H. grosseserratus*/P 21
  - Amp *H. angustifolius*/P 21
- $F_1$  progeny of hexaploid *H. californicus*/HA 89 ( $2n=68$ )



## *Rf* identification

CMS 514A (2n=34) × six materials (2n=68)

Male-fertile (MF) F<sub>1</sub> (2n=51) × HA 89

MF BC<sub>1</sub>F<sub>1</sub> × HA 89

BC<sub>2</sub>F<sub>1</sub> (2n=34-39)

× HA 89, HA 821,  
or SIB

MF (2n=35) × HA 89

G07/553 (2n=34, CMS)

G07/517 (2n=34, MF)

G07/610 (MF)

G07/612 (MF)

G07/623 (MF)

CMS 514A ×

F<sub>1</sub> for genetic mapping

G08/598

G08/613

G08/621

Test F<sub>2</sub> population

F<sub>2</sub> mapping population

Test F<sub>2</sub> population

## *Rf* introgression and inheritance

## Molecular mapping and characterization

- Mitotic chromosome counting and genomic *in situ* hybridization (GISH) analysis
- Test populations: derived from G08/598, G08/613, and G08/621

Pedigree: CMS 514A/6/(CMS 514A//Amp *H. angustifolius*/P 21/3/2\*HA 89/4/HA 821/5/HA 89 and Self) SIB)

Mapping population: derived from  
G08/613

- Molecular marker screening

  - Bulked segregant analysis (BSA)

    - Bulk F: a homozygous fertile  $F_2$  bulk

    - Bulk S: a sterile  $F_2$  bulk

    - Bulk  $2n=35$ : a fertile  $BC_4F_2$  bulk with  $2n=35$

    - Bulk  $2n=34$ : a sterile  $BC_4F_2$  bulk with  $2n=34$

370 pairs of SSR primers

65 SSR and 28 EST-SSR markers from the candidate LG 3 of 23 maps in the Sunflower CMap Database

- Statistical analysis and linkage map construction

Chi-square test

MAPMAKER/Exp version 3.0b (Lander *et al.* 1987)

# Results and Discussion

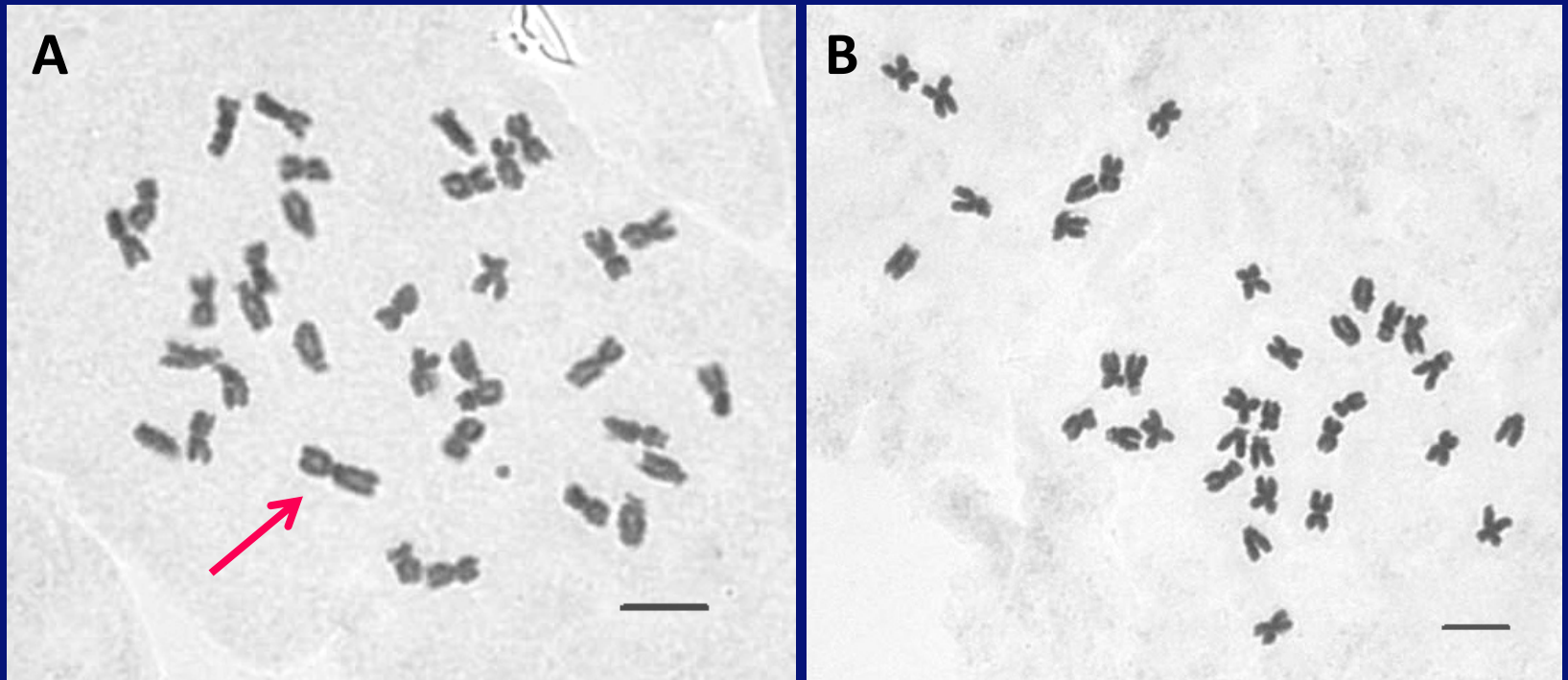
- Identification of the *Rf* gene in Amp *H. angustifolius*/P 21, which was designated *Rf*<sub>6</sub>

Cross	Plant No.	Fertile plant No.
514A // Amp <i>H. angustifolius</i> / P 21	13	13
514A // Amp <i>H. grosseserratus</i> / P 21	10	0
514A // <i>H. californicus</i> / HA 89	4	0
514A // Amp of <i>H. atrorubens</i> / HA 89	6	0
514A // Amp of <i>H. mollis</i> / P 21	11	0
514A // Amp of <i>H. cusickii</i> / P 21	6	0

○ Genetic analysis of the alien chromosome carrying  $Rf_6$

The testcross and selfed progenies of MF plants ( $2n=35$ )

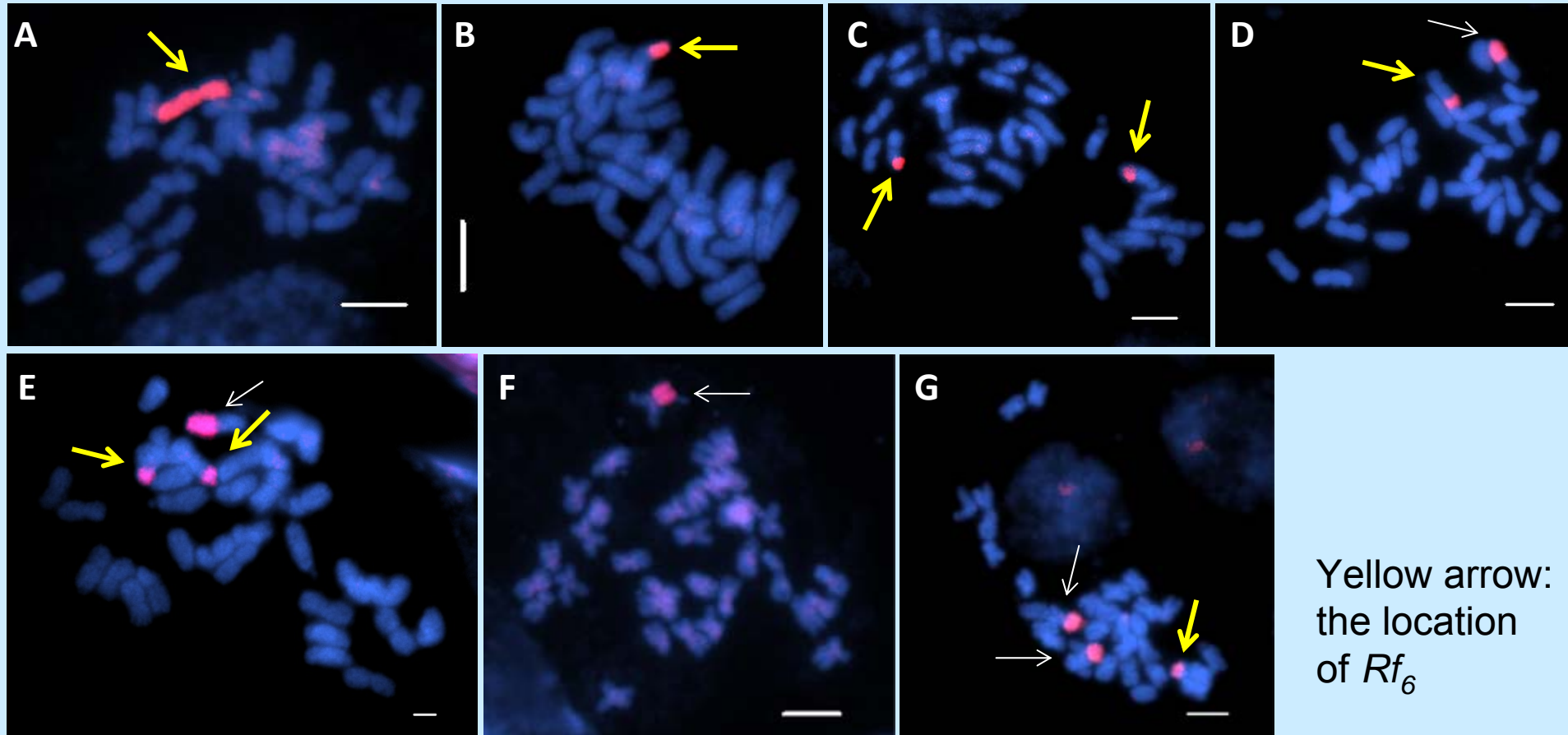
Cross	Total	2n=34	2n=35	2n=35	2n=36	2n=36
	plants	MF:MS	MF:MS	(%)	MF:MS	(%)
514A x (G07/596 and G07/598) (MF, $2n=35$ )	59	0:55	4:0	6.8		
G09/2614 (MF, $2n=35$ ) x HA 89	59	0:39	20:0	33.9		
G08/638 and G08/672 (MF, $2n=35$ ) selfed- total	116	1:75	25:10	30.2	4:1	4.3
G08/638 (MF, $2n=35$ ) selfed (1)	57	1:36	8:9	29.8	2:1	5.3
G08/672 (MF, $2n=35$ ) selfed (2)	59	0:39	17:1	30.5	2:0	3.4



Comparison of chromosome spreads of male-fertile (MF) ( $2n=35$ ) (A) and male-sterile (MS) ( $2n=34$ ) (B) plants.

The arrow shows the larger chromosome compared to other chromosomes, which is assumed to be the alien chromosome from *H. angustifolius* carrying *Rf*<sub>6</sub>. Bars=5  $\mu\text{m}$ .

# ○ Mitotic GISH and cytogenetic analyses

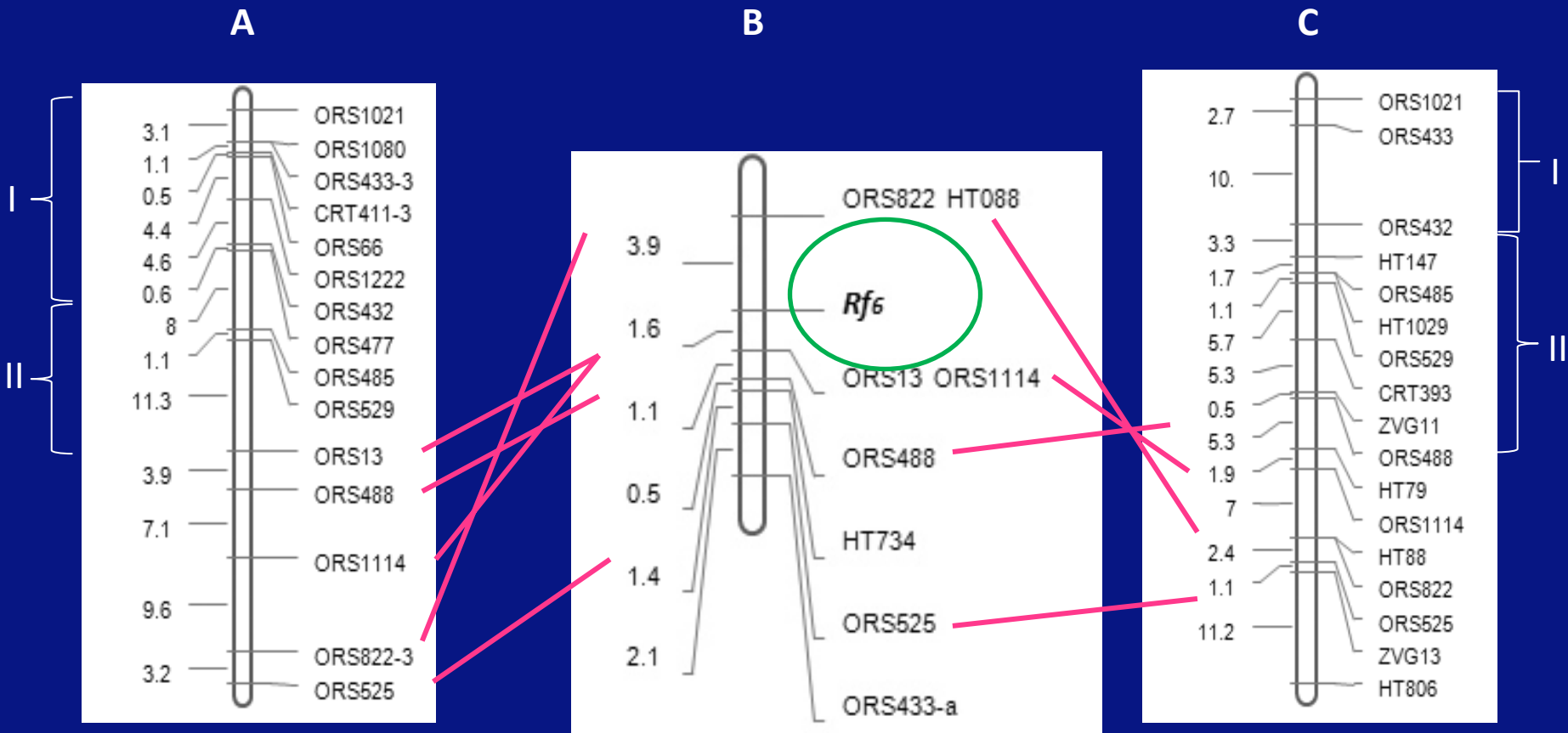


**A**) a heterozygous MF plant, G08/638 (2n=35); **B**) a heterozygous MF plant (2n=34) derived from G08/613; **C**) a homozygous MF plant (2n=34) derived from G08/613; **D**) a heterozygous MF plant (2n=34) derived from G08/621; **E**) a homozygous MF plant (2n=34) derived from G08/598; **F**) an MS plant (2n=34) derived from G08/621; **G**) an abnormal plant derived from G08/621. Bars=5  $\mu$ m.



# ○ Fertility segregation and mapping of *Rf*<sub>6</sub> on LG 3

Traits or markers	Number of F <sub>2</sub> plants	Observed number <sup>a</sup>				Ratio expected	$\chi^2$	P-value
		A	H	B	C			
<i>Rf</i> <sub>6</sub> <sup>b</sup>	255	89			166	1:3	13.33	2.6 x 10 <sup>-4</sup>
<i>Rf</i> <sub>6</sub> <sup>c</sup>	221	89	113	19		1:2:1	44.46	2.2 x 10 <sup>-10</sup>
ORS822	220	85			135	1:3	21.82	3.0 x 10 <sup>-6</sup>
HT088	220	85	118	17		1:3	43.20	4.9 x 10 <sup>-11</sup>
ORS433-a	220	82			138	1:3	17.67	2.6 x 10 <sup>-5</sup>
ORS13	220	83	118	19		1:2:1	38.40	4.6 x 10 <sup>-9</sup>
ORS1114	220	83	118	19		1:2:1	38.40	4.6 x 10 <sup>-9</sup>
HT734	220	82	119	19		1:2:1	37.55	7.0 x 10 <sup>-9</sup>
ORS488	220	82	119	19		1:2:1	37.55	7.0 x 10 <sup>-9</sup>
ORS525	221	86			135	1:3	22.82	1.8 x 10 <sup>-6</sup>
ORS433-b <sup>d</sup>	216	58			158	1:3	0.40	0.53



**Figure .** The position of the fertility restoration gene *Rf<sub>6</sub>* on LG 3 of the sunflower map. **A)** A partial map of LG 3 of Tang *et al.* (2003); **B)** Mapping result of *Rf<sub>6</sub>* on LG 3, using 221 *F<sub>2</sub>* plants; **C)** A partial map of LG 3 of RHA 280 x RHA 801\_RIL (in press). Region I indicates the region is not linked to *Rf<sub>6</sub>*, and Region II indicates the region where the possible break point of the translocation with *Rf<sub>6</sub>* is located.

The order of the markers were reversed.

# Summary

- ◉ An *Rf* gene, *Rf<sub>6</sub>*, was identified to restore the male fertility of CMS 514A, originated from *H. angustifolius*
- ◉ *Rf<sub>6</sub>* was introgressed into cultivated sunflower. It was located on a small chromosomal translocation
- ◉ *Rf<sub>6</sub>* was located on LG 3 of the sunflower SSR map, with 8 linked markers in a mapping population. The markers ORS13 and ORS1114 were 1.6 cM away

- Severe segregation distortions were observed for both the fertility trait and the linked markers, suggesting the possibility of a low frequency of recombination or gamete selection in this region
- This study discovered a new CMS/Rf gene system and provided significant insight into the genetic basis of this system
- This will diversify the germplasm for sunflower breeding and facilitate understanding of the interaction between the cytoplasm and nuclear genes

# Acknowledgments

- Lisa A. Brown
- Ridhima Katyal
- Jordan Hogness
- Alexis Ganser
- Yuni Chen
- Marjorie A. Olson
- Dr. Larry G. Campbell
- Dr. Lili Qi
- Dr. Prem P. Jauhar
- Dr. Steven S. Xu
- Dr. Brady A. Vick
- Dr. Chengsong Zhu (Kansas State University)
- Dr. Wentao Li (University of California-Davis)
- Dr. Zahirul Talukder (North Dakota State University)
- Dr. Yunming Long (North Dakota State University)