

Competitive Research Grant

Sub-Project Completion Report

On

Screening of Shattering Tolerance of *Brassica Napus*

Project Duration

May 2016 to September 2018

Regional Agricultural Research Station
Bangladesh Agricultural Research Institute
Jamalpur



Submitted to
Project Implementation Unit-BARC, NATP-2
Bangladesh Agricultural Research Council
Farmgate, Dhaka-1215



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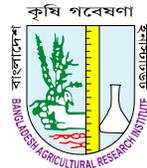
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Citation:

Screening of Shattering Tolerance of *Brassica Napus*
National Agricultural Technology Program-Phase II Project (NATP-2)
Bangladesh Agricultural Research Council (BARC)
New Airport Road, Farmgate, Dhaka – 1215, Bangladesh

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Bangladesh

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Published in: September 2018

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Acronyms

BARC - Bangladesh Agricultural Research Council

CRG - Competitive Research Grant

GoB - Government of Bangladesh

Ha - Hectare

Kg - Kilogram

LoA - Letter of Agreement

N/A - Not Applicable

NATP - National Agricultural Technology Project

PCR - Project Completion Report

PI - Principal Investigator

PIU - Project Implementation Unit

PP - Project Proposal

PSO - Principal Scientific Officer

RARS - Regional Agricultural Research Station

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Executive Summary

Brassica napus has emerged as an important cultivated oilseed crop species in the world. *B. napus* is high yield potential and wide adaptable. Its cultivation in Bangladesh is very limited due to pod shattering in mature stage. Dehiscence of siliqua due to external forces at maturity leads to siliqua shatter results in significant yield loss. It is needed for substantial improvement in shatter resistance to prevent significant seed loss at mature. A research was carried out for screening of shattering tolerance of *Brassica napus* at Regional Agricultural Research Station, Jamalpur during May, 2017 to June, 2018. The lines BEN-21, BEN-19 and NAP-16046 are tolerance to pod shattering at mature stage. Grain yield of these lines is poor. NAP-0733-1 produced the highest grain yield which was 19.6% higher than check variety. NAP-0733-1, NAP-15020 and NAP-16041 produced high yield. They were also moderately tolerance to pod shattering. Compensating yield and shattering tolerance; NAP-15020, NAP-16041 and NAP-0733-1 may be selected as shattering tolerance.

CRG Sub-Project Completion Report (PCR)

A. Sub-project Description

1. Title of the CRG sub-project: **SCREENING OF SHATTERING TOLERANCE OF *BRASSICA NAPUS***

2. Implementing organization: Bangladesh Agricultural Research Institute (BARI)

3. Name and full address with phone, cell and e-mail of PI: **Dr. Md. Manjurul Kadir**, Principal Scientific Officer, RARS, BARI, Jamalpur, Phone: 0981-64395 (Office), 0981-64628 (Residence), Cell Phone: 01717054128, e-mail: mkadirbari@yahoo.com

4. Sub-project budget (Tk):

4.1 Total: 784082/-

4.2 Revised: 657331/-

5. Duration of the sub-project:

5.1 Start date (based on LoA signed): May 2017

5.2 End date : September, 2018

6. Justification of undertaking the sub-project:

Bangladesh has been suffering from acute shortage of edible oil. Domestic production can hardly meet up 25-30% of national requirement. She imported 1.8 million tons of edible oil at a cost of 3413.36 million US dollar (Anonymous, 2018). Locally, oilseed production rose 10.67% to 0.934 million tons in 2014-15 from a year earlier. The domestic production is rising but still much lower than demand. Rapeseed-mustard oil is the main source of the edible oil in the country and covered 75% oilseed area. Among the oiliferous Brassicas, mainly the varieties of *B. rapa* is being cultivated in the country. Its yield potentiality is low and less responsive to high management compared to *B. rapa*

B. napus is a new introduced crop in Bangladesh. Bangladesh Agricultural Research Institute (BARI) and Bangladesh Institute of Nuclear Agriculture (BINA) has developed some varieties of *B. napus*. Its yield potentiality is quite high and responsive to high management. Cultivation of *B. napus* may be a scope to increase local production and to decrease import of edible oil. But seed shattering or pod shedding after maturity is a major concern in *B. napus* production. Shattering can cause up to 50% yield loss if harvesting is delayed due to adverse condition (Price *et al.*, 1996). Shredder seeds persist in the soil giving rise to volunteer plants and increase weed control problem in the subsequent years. Volunteer *Brassica* plants may cause phototoxic effect on subsequent crops also (Vera *et al.*, 1987). Harvest before full maturity may reduce yield loss from shattering, but it reduce seed quality due to higher seed chlorophyll content

(Vera *et al.*, 2007). Cultivation of high yield potential Brassica like *B. napus* is essential to increase domestic production. Shattering tolerance *B. napus* is essential to reduce yield loss.

Though *Brassica napus* is an important and high yield potential edible oil crop but it is subject to significant losses in seed production as a result of premature shattering before and during harvest. Seed shedding from pods is commonly referred to as pod shatter, which is a major production risk in *Brassica napus* worldwide. Large scale cultivation of *Brassica napus* may help to reduce import edible oil from abroad and increase local production but its cultivation is confined to little area near research station. Farmers are not interested to grow it for its yield loss from shattering. Very little research work has been done to develop shattering tolerant *Brassic napus*. Raman *et al.*, 2014 opined that shattering resistance genes in *Brassic napus* is limited; many of the genes that might control this trait were not included during the natural creation, or were retained during domestication and selection process. They mentioned that breeders will need to target introduction of useful genes from related species. To find out shattering tolerant *Brassica napus* is crucial for increasing yield and local production of oilseed in Bangladesh. Therefore, screening of shattering tolerance *Brassica napus* is necessary for accelerating oilseed improvement in this country.

Acute shortage of edible oil has been prevailing in Bangladesh since British territory. Every year, the edible oils demand in Bangladesh has been met up by importing of the crude soybean and palm oil. In contrast, locally produced rapeseed and mustard along with sesame seed, linseed and groundnut seed are contributed very negligible in the total demand of the edible oils. The values of imported edible oils and oilseeds were USD 3413.36 million dollars in 2017-2018, respectively (Anonymous, 2018). The Perspective Plan of Bangladesh (PPB) 2010-21 aimed at increasing the production of domestic oil seeds for providing 40 g/day (14.6 kg/ year) to the population of edible oil in 2021 at page 81 of PPB as against 57.43 gm (20.96 kg per year) advised by FAO. Population would be reach to the 17.06 cores in 2021 as estimated with the population increase rate of 1.32%. As per PPB the demand for edible oil would be needed up to 24.91 lac MTs in 2021. In Bangladesh, presently on average 9.70 kg/ annum or 26.57 g/ day oilseed is consumed by the people. On contrary, in Pakistan and India peoples are consumed 14 kg and 13.92 kg/annum, respectively.

Rapeseed-mustard is the major contributor for edible oil production. The country can provide only 25-30% of national requirement. The seed yield of the rapeseed-mustard varieties cultivated in the country is poor. The reasons for low yield are partly cultivation of low yielding

varieties of *B.rapa* and partly poor agronomic-management of the crop. Among the oilseeds Brassicas, *B. napus* is the most productive. Its pod and seed size is large and yield is much higher than *B rapa*. Normally, *B rapa* is less responsive to high management but *B napus* produce higher yield under high management. It can tolerate water logging to some extent. Disease and insect infestation is less compared to *B rapa*. Shattering during harvest is the major concern for *Brassica napus* varieties developed in the country. Shattering resistance/ tolerance is an important trait for napus improvement in Bangladesh as crop ripens and harvest in dry condition. Yield can be lost 50% due to shattering. Find out of shattering tolerance *B. napus* will accelerate its cultivation and increase yield. Screening for identify shattering resistance Brassicanapus lines which were developed from crossing and back crossing with *Brassica juncea* is necessary.

There is very little works has been done to develop *B. napus* variety which are tolerant to shattering. In China (Wang *et al.*, 2007) screened *B. napus* for shattering resistance and found some lines were tolerance to shattering which were derbies from the crosses between *B napus* and *B Juncea*. Genetic information conferring non- shattering of silique has been introgressed in *Brassica napus* following; interspecific hybridization between *Brassica juncea* and *B. napus* and reconstituted *B. napus* plant with complete non-dehiscence of its fruits was isolated in the backcross generation (Prakash and Chopra, 1988). Bangladesh Agricultural Research Institute (BARI) and Bangladesh Institute of Nuclear Agriculture (BINA) has released 8 high yielding varieties of *Brassica napus* (BARI released 4 & BINA 4). In Regional Agricultural Research Station, Jamalpur, Some lines of *Brassica napus* has been developed through crossing and back crossing between *Brassica napus* and *Brassica juncea* to incorporate shattering resistance in *Brassica napus*.

7. Sub-project goal: Increase yield of rapeseed

8. Sub-project objectives: (a) Development of shattering tolerance *B napus*lines

(b) Increase the yield of *B. napus*

9. Implementing location: Regional Agricultural Research Station (RARS), Jamalpur

10. Methodology in brief: Resistance to shatter is important trait for rapeseed improvement in Bangladesh and most of the world, because as the crop ripens and is harvested under dry condition. Pods of *Brassica napus* shatter during maturity and harvesting resultsof marked losses of seed. The field was well plough done and laddered by a power tiller to pulverize the cloud and to remove the weeds from the field. The field was spited into different plots. Twenty five

lines of *B. napus* developed through back crossing between *B. napus* and *B. juncea* and BARI Sharisa-8 as check were used in the experiment. These lines were laid out in randomized complete block design with three replications at Regional Agricultural Research Station, Jamalpur during Rabi, 2017-2018. Plot size was 3.0 x 2.10 m. Recommended manures and fertilizers (260, 170, 90, 160, 5 & 10 kg/ha urea, TSP, MOP, Gypsum, Zn oxide & Boric acid) were applied. Half of urea and all other fertilizers and manure were mixed together and were applied as basal dose in the field just before final plough. Application of basal doses on the soil, one plough followed by one ladder was done to well mix up of fertilizers and manure. Seeds of different lines were sown on the 14th of November, 2017 in the plot 30cm apart row continuously. The remaining half urea was applied at the time of flower initiation. Weeding and thinning was done at the age of 12 days after sowing. First irrigation was applied at the age of 20 days after sowing and just before flower initiation. Second irrigation was applied at the age of 50 days after sowing and just after completion of maximum flowering. Roval @2gm/litre water was applied to control Alternaria leaf spot at the age of 55 days and 63 days after sowing.

Data has been recorded on plant height, no. pods/plant, no. seed/pod 1000 seed weight, days to maturity and yield/plot. Shattering tolerance was measured by striking the mature pod/siliquea by a small iron ball. Mature pods/ siliquea was put in a still box in which two iron balls were kept. The box was set up on an electric stirrer which moved for 30seconds by 150 rpm. After stirring 30 seconds; number of shattered and non shattered pods were counted and it was expressed in percentage.

A shattering resistance index (SRI) was prepared by following the above mention process. The SRI was calculated from a given sample, number of non shattered pods divided by total number of pods, multiplying by 100. High SRI bearing lines was selected as shattering tolerance. Shattering tolerance lines was selected. Data were analyzed statistically and report was prepared and submitted to Bangladesh Agricultural Research Council (BARC) through proper channel.



Shattered Brassica napus



Brassica napus pod shatter measuring device

11. Results and discussion:

Brassica napus is an important edible oilseed produced crop. Its yield potentiality is very high but it has the tenancy to shatter at harvest. The performance of *Brassica napus* lines has been given in table-1.

The table-1 revealed that plant height of all lines was below 84.0cm indicating dwarf type of plants was produced by the evaluated *Brassica napus* lines. The tallest plant was recorded from NAP-14078 and the dwarf from NAP-0865. The range of plant height was from 68.3cm to 82.0 cm. The highest number of primary branches/plant was recorded from NAP-0865 and the lowest number from BARI Sharisa-13. The highest number of secondary branches/plant was produced by NAP-16064 and NAP-16090 and the lowest was recorded from BARI Sharisa-13. The highest number of pods/plant was produced by NAP-14036 and the second and third highest was recorded from NAP-16025 and NAP-14054. The highest number of seeds/pod was recorded from NAP-16090. The second highest number of seeds/pod was recorded from NAP-16066 and the third highest from NAP-0733-1. NAP-16090 produced the longest pod followed by NAP-16068 and NAP-1661. The highest 1000 seed wt(g) was recorded from NAP-14036. The second 1000 seeds weight was recorded from NAP-16068, NAP-14037 and BEN-21. All the lines and varieties took less than 85 days to mature. The NAP-16064, NAP-16068, NAP-0865, NAP-16061, NAP-16066 and NAP-16025 took 80 days to mature. The highest yield was produced by NAP-0733-2. It produced 2084 kg/ha yield which was 19.6% higher yield than check variety BARI Sharisa-8. The second highest yield was recorded from the line NAP-16082. It produced 15.5% higher yield than BARI Sharisa-8. The third highest yield was recorded from NAP-14036. It produced 1992 kg/ha which was 14.3% higher the check variety BARI Sharisa-8. The shattered and non shattered pods were measured at 22%-24% moisture. It was found that the highest non shattered pods were recorded from BEN-21 and it was 52%. The second highest non shattered pods were recorded from BEN-19 and the third was from NAP-16046 and NAP-15020.

The *Brassica napus* line, NAP-0733-1 produced the highest yield and simultaneously its % non shattered pods were 32. It indicates that this line is not only produce good yield but also moderately tolerance to shattering. Similarly, NAP-15020 and NAP-16041 produced good yield and they were also moderately tolerance to shattering as their % non shattering pods were 34 and 33.3 respectively.

On the other hand, the highest % non shattering pods were recorded from BEN-21 which was 52%. It indicates that this line has the capability to tolerance shattering. But its yield was very

low. It produced only 1268 kg/ha grain yield which was 27% lower than the existing check variety BARI Sharisa-8. BEN-19 also moderately tolerance to shattering but it produced 23% lower yield than Check. Similarly, NAP-14077 produced the lowest yield among the lines screened under this experiment. It produced only 1043 kg/ha grain yield which was 40.1% lower than check variety. But its % non shattering pods were 33.3 indicates that this line is moderately tolerance to pods shattering during harvest.

Conclusion: The highest % non shattering pods were recorded from BEN-21 and BEN-19 but their yield potentiality is very poor. These lines may be select as shattering tolerance and may be used in further breeding program to incorporate this trait to high yielding varieties/ lines. Compensating yield and shattering tolerance; NAP-15020, NAP-16041 and NAP-0733-1 may be selected. They were moderately shattering tolerance as well as, produced good yield.

Table-1.Performance of *Brassica napus* lines under screening of shattering tolerance experiment

Variety	Plant Height (cm)	Pry. Branches/ plant	2 nd branches/ plant	No.pods/ plant	No. seed/ pod	Length of Pod (cm)	1000 Seed Weight (g)	Duration (Days)	Yield (kg/ha)	% yield Increase Over Check	Shattered (%)	Non Shattered (%)
NAP-16001	75.5	2.5	0.8	62.7	21.6	7.50	4.2	83	1747	0.27	66.7	33.3
NAP-16064	69.8	2.6	2.2	70.8	20.6	7.50	4.0	80	1490	-14.4	76.0	24.0
NAP-16068	72.2	2.8	1.7	50.8	21.6	7.90	4.4	80	1512	-13.2	76.7	23.3
NAP-16081	72	3.1	1.3	64.2	25.5	7.80	4.2	82	1334	-23.4	81.3	18.7
NAP-16082	74.7	3.3	1.3	59.4	23.4	7.10	3.6	83	2013	15.5	82.7	17.3
NAP-16090	75.7	3.3	2.2	62.1	27.3	8.05	3.4	82	1572	-9.70	76.0	24.0
BEN-19	75.6	2.5	1.7	60.8	24.4	7.12	4.0	81	1341	-23.0	65.0	35.0
NAP-0717-2	78.4	3.1	2.1	58.6	24.3	6.90	4.0	83	1756	0.79	67.7	33.3
NAP-0733-1	81.0	2.7	2.0	53.4	25.9	7.10	4.2	82	2084	19.6	68.0	32.0
NAP-0865	68.3	3.0	1.4	51.6	24.6	7.34	3.2	80	1406	-19.2	70.7	29.3
NAP-0876	79.2	3.4	2.0	62.4	25.5	7.30	2.6	83	1466	-15.8	69.3	30.7
NAP-14077	78.1	2.7	1.6	69.1	24.3	7.60	2.4	81	1043	-40.1	67.7	33.3
NAP-14078	82.0	2.6	1.5	52.0	24.0	7.40	2.2	83	1546	-11.2	74.7	25.3
NAP-16041	78.2	2.3	0.9	63.6	22.2	7.50	2.7	83	1973	13.2	85.3	14.7
NAP-16046	79.4	2.5	0.9	65.2	24.1	7.60	2.6	83	1673	-3.96	66.0	34.0
NAP-16061	70.8	2.7	1.6	59.1	23.3	7.90	2.4	80	1446	-16.9	69.3	30.7
NAP-16066	76.1	2.7	1.2	63.3	27.1	7.80	2.4	80	1458	-16.3	84.0	16.0
NAP-16084	77.6	2.6	0.9	59.3	25.0	7.50	2.6	83	1636	-6.09	80.0	20.0
NAP-16025	79.7	3.3	1.7	74.7	24.1	7.60	4.4	80	1679	-3.62	72.0	28.0
NAP-15020	75.1	2.5	1.0	68.03	23.4	7.60	4.0	83	1991	14.2	66.0	34.0
NAP-14002	74.0	3.2	1.4	67.5	22.9	6.80	3.6	82	1910	9.63	73.3	26.7
NAP-14036	75.0	2.8	1.0	77.7	23.5	7.10	4.8	81	1992	14.3	72.0	28.0
NAP-14037	77.4	2.8	1.7	65.7	23.6	6.90	4.4	81	1585	-9.02	72.7	27.3
NAP-14054	74.7	3.0	1.7	71.5	23.9	7.05	4.0	82	1545	-11.3	78.7	21.3
BEN-21	81.3	2.7	1.4	65.7	23.7	7.20	4.4	84	1268	-27.2	48.0	52.0
BARI-8	79.9	2.7	1.3	62.3	24.9	7.00	4.0	84	1740	-0.12	75.7	25.3
BARI-13	79.5	2.2	0.0	49.2	25.0	6.70	3.6	85	1865	7.05	73.7	26.3
CV(%)	12	9.5	5.6	9.7	5.3	6.2	4.5	6.0	8.7	-	11.2	10.5
Sign. level	*	*	**	*	**	**	**	**	*	-	*	*

12. Research highlight/findings:

(i) The highest yield was produced by NAP-0733-2. It produced 2084 kg/ha yield which was 19.6% higher yield than check variety BARI Sharisa-8.

(ii) The highest non shattering pods were recorded from BEN-21, NAP-16046 and NAP-15020 but their yield was not satisfactory. These lines may be select as shattering tolerance and may be used in further breeding program to incorporate this trait to high yielding varieties/ lines.

(iii) Compensating yield and shattering tolerance; NAP-15020, NAP-16041 and NAP-0733-1 may be selected. They were moderately shattering tolerance as well as, produced good yield.

B. Implementation Position

1. Procurement: Not applicable

Description of equipment and capital items	PP Target		Achievement		Remarks
	Phy (#)	Fin (Tk)	Phy (#)	Fin (Tk)	
(a) Office equipment					
(b) Lab & field equipment					
(c) Other capital items					

2. Establishment/renovation facilities: Not applicable

Description of facilities	Newly established		Upgraded/refurbished		Remarks
	PP Target	Achievement	PP Target	Achievement	

3. Training/study tour/ seminar/workshop/conference organized: Not applicable

Description	Number of participant			Duration (Days/weeks/ months)	Remarks
	Male	Female	Total		
(a) Training	-	-	-	-	
(b) Workshop	-	-	-	-	

C. Financial and physical progress

Fig in Tk

Items of expenditure/activities	Total approved budget	Fund received	Actual expenditure	Balance/ unspent	Physical progress (%)	Reasons for deviation
A. Contractual staff salary	145832	145832	134965	10867	92.54	Time constraint
B. Field research/lab expenses and supplies	493000	449671	4,13,844	35735	92.03	Do
C. Operating expenses	66200	51,744	51,744	00	100	
D. Vehicle hire and fuel, oil & maintenance	10000	10084	10084	00	100	
E. Training/workshop/seminar etc.	34050	00	00	0	00	
F. Publications and printing	35000	00	00	00	00	Do
G. Miscellaneous	00	00	00	00	00	
H. Capital expenses	00	00	00	0	00	

D. Achievement of Sub-project by objectives: (Tangible form)

Specific objectives of the sub-project	Major technical activities performed in respect of the set objectives	Output(i.e. product obtained, visible, measurable)	Outcome(short term effect of the research)
(a) Development of shattering tolerance <i>B napus</i> lines	An experiment was carried out with 26 lines of brassica napus to find out shattering tolerance lines at RARS Jamalpur. A shattering resistance index (SRI) was prepared by striking the mature pod/siliquea by a small iron ball. Mature pods/siliquea was put in a still box in which two iron balls was kept. The box was set up on an electric stirrer which moved for 30seconds by 150 rpm. After stirring 30 seconds; number of shattered and non shattered pods were counted and it was expressed in percent age.	Three lines of Brassica napus BEN-21, NAP-16046 and NAP-15020 were identified as shattering tolerance.	Yield loss due to shattering of Brassica napus will be reduced.
(b)) Increase the yield of <i>B. napus</i>	An experiment on Screening for shattering tolerance of Brassica napus was conducted	NAP-0733-2, NAP-16082 and NAP-14036 were found higher yielder.	These lines may be grown for higher

Specific objectives of the sub-project	Major technical activities performed in respect of the set objectives	Output(i.e. product obtained, visible, measurable)	Outcome(short term effect of the research)
	at RARS, Jamalpur. High yield potential lines with shattering tolerance were identified.		yield.
(c) Reduce the shattering loss in <i>B. napus</i>	An experiment on Screening for shattering tolerance of Brassica napus was conducted at RARS, Jamalpur and pod shattering tolerance lines were identified using shattering resistance index (SRI).	NAP-15020, NAP-16041 and NAP-0733-1 were found higher yielder and tolerance to shattering.	NAP-15020, NAP-16041 and NAP-0733-1 will reduce shattering loss.

E. Materials Development/Publication made under the Sub-project:

Publication	Number of publication		Remarks (e.g. paper title, name of journal, conference name, etc.)
	Under preparation	Completed and published	
Technology bulletin/ booklet/leaflet/flyer etc.	-	-	
Journal publication	-	-	
Information development	-	-	
Other publications, if any Different print media		-	

F. Technology/Knowledge generation/Policy Support (as applied):

i. Generation of technology (Commodity & Non-commodity)

1. BEN-21, NAP-16046 and NAP-15020 are tolerant to shattering
2. NAP-0733-2 is high yield potential.
3. NAP-15020, NAP-16041 and NAP-0733-1 high yielder and moderately shattering tolerant.

ii. Generation of new knowledge that help in developing more technology in future

BEN-21, NAP-16046 and NAP-15020 are tolerant to shattering but low yielder. These lines may be used for incorporation non shattering gene to high yielding variety.

iii. **Technology transferred that help increased agricultural productivity and farmers' income**

High yield potential lines with shattering tolerant may be released as variety. Farmers will be benefited by reducing shattering loss of *Brassica napus* increase yield.

iv. **Policy Support:** Market price volatilization due to shortage of edible oil in Bangladesh will be controlled.

G. Information regarding Desk and Field Monitoring

i) **Desk Monitoring [description & output of consultation meeting, monitoring workshops/seminars etc.):**

A seminar on “Research Progress” was held on 26.02.2018 arranged by PIU, BARC, NATP-2 at BARC. Detail discussion about research progress was done in that seminar. A workshop on “Monitoring and Evaluation of Research Activities” was held on 15.05.2018 at BARC, Dhaka. Another “Annual Research Review Workshop” was held on 12.09.2018 at BARC, Farmgate, Dhaka. Through this desk monitoring, the research activities of project was improved and different lacking were recovered.

ii) **Field Monitoring (time& No. of visit, Team visit and output):** Internal field monitoring at research plots were done 2 times on 25.02.2018 and 28.02.2018 by (1) Chief Scientific Officer, RARS, Jamalpur and all other scientists of RARS, BARI, Jamalpur (2) Director Oilseed Research Centre, BARI, Joydebpur, Gazipur. Field visit improved research activities through open discussion and it helped to collect data properly.

H. Lesson Learned (if any)

i) Interspecific hybrid development of *Brassica napus* and *Brassica juncea* is very difficult as cross success is limited. Data recording for shattering resistance index (SRI) in particular moisture of pods is tough.

I. Challenges (if any)

Brassica napus is high yield potential. Farmers are less interested to grow *Brassica napus* due to pod shattering loss at mature. It is also less familiar to the farmers.

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Signature of the Principal Investigator

Date

Seal

Counter signature of the Head of the

organization/authorized representative

Date

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