Screening of germplasm and elite lines for machine harvestability traits,

Activity 1: Evaluation of performance of lentil genotypes for machine harvestability

<table>
<tr><td>Title</td><td>:</td><td>Evaluation of performance of lentil genotypes for machine harvestability</td></tr>
<tr><td>Objectives</td><td>:</td><td>Identify the lentil genotypes suitable for machine harvesting
Evaluate the lentil machine harvestable genotypes for harvest losses and efficiencies</td></tr>
<tr><td>Activities</td><td>:</td><td>Continued</td></tr>
<tr><td>Expected outcomes</td><td>:</td><td>Genotypes suitable for machine harvesting developed
Harvesting losses and efficiency of genotypes under machine and manual harvesting determined</td></tr>
<tr><td>Observations to be taken</td><td>:</td><td>Crop phenology
Growth and yield attributes
Yields and harvest index
Harvest losses
Harvesting efficiencies</td></tr>
<tr><td>Genotypes</td><td>:</td><td>
<table>
<tr><td>010S 96131-2</td><td>2009S 96568-1</td><td>2007S 96803-3</td><td>09S 83183-01</td><td>2009S 96510-8</td></tr>
<tr><td>010S 96134-3</td><td>2009S 96102-7</td><td>2007S 96803-5</td><td>09S 83191-04</td><td>2009S 96511-3</td></tr>
<tr><td>06S 53110-02</td><td>2009S 96501-5</td><td>2007S 96808-1</td><td>09S 83192-01</td><td>2009S 96537-1</td></tr>
<tr><td>010S 96130-1</td><td>2009S 96518-1</td><td>2007S 96811-8</td><td>2009S 96501-2</td><td>2009S 96549-2</td></tr>
<tr><td>010S 96143-4</td><td>2009S 96518-2</td><td>2009S 96101-2</td><td>2009S 96502-12</td><td>ILL4400</td></tr>
<tr><td>010S 96155-2</td><td>2009S 96101-5</td><td>2009S 96502-13</td><td>2009S 96505-2</td><td>ILL4401</td></tr>
<tr><td>06S 53110-03</td><td>2009S 96574-5</td><td>2009S 96511-1</td><td>2009S 96505-3</td><td>ILL5883</td></tr>
<tr><td>010S 96146-4</td><td>2009S 96575-10</td><td>2009S 96573-3</td><td>2009S 96506-1</td><td>ILL5888</td></tr>
<tr><td>08S 40111-01</td><td>2009S 96575-6</td><td>2009S 96575-17</td><td>2009S 96510-3</td><td>Bakaria</td></tr>
<tr><td>08S 40106-01</td><td>2007S 96803-2</td><td>2009S 96574-3</td><td>2009S 96510-7</td><td>L-24</td></tr>
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</td></tr>
<tr><td>Key outcomes</td><td>:</td><td>Plants were erect to semi-erect and grown tall up to 33 cm and produced first pods at the average height of 16 cm. The machine harvestable genotypes recorded lower pod shattering, pod drop and lodging susceptibility than local check, Bakria. Some promising lines identified for machine harvesting is presented in the Table 1.

Table 1: Promising lines identified for machine harvesting
<table>
<tr><th>SN</th><th>Entry</th><th>Plant height</th><th>Pod height</th><th>Early growth vigour</th><th>Pod shattering</th><th>Pod drop</th><th>Lodging Susceptibility</th></tr>
<tr><td>1</td><td>09S 83183-01</td><td>32.6</td><td>20.7</td><td>3</td><td>3</td><td>3</td><td>3</td></tr>
<tr><td>2</td><td>2009S 96573-3</td><td>31.8</td><td>19.7</td><td>3</td><td>3</td><td>3</td><td>2</td></tr>
<tr><td>3</td><td>2007S 96811-8</td><td>31.5</td><td>16.8</td><td>2</td><td>2</td><td>3</td><td>3</td></tr>
<tr><td>4</td><td>010S 96143-4</td><td>30.8</td><td>16.5</td><td>3</td><td>3</td><td>3</td><td>3</td></tr>
<tr><td>5</td><td>2009S 96505-3</td><td>30.8</td><td>18.0</td><td>3</td><td>3</td><td>3</td><td>0</td></tr>
<tr><td>6</td><td>2009S 96102-7</td><td>30.7</td><td>18.2</td><td>3</td><td>3</td><td>2</td><td>0</td></tr>
<tr><td>7</td><td>2009S 96518-2</td><td>30.3</td><td>19.2</td><td>3</td><td>3</td><td>3</td><td>3</td></tr>
</table>
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		8	010S 96155-2	30.3	17.0	2	3	4	3
		9	09S 83191-04	30.2	17.3	3	3	4	3
		10	2009S 96518-1	29.7	17.3	2	3	3	0
		11	Bakria	26.8	15.0	3	3	5	6

Activity 2: Genome Wide Association Studies (GWAS) for traits to facilitate machine harvesting and other economically important traits (tolerance to biotic and abiotic stresses) to improve crop yields in lentil.

Title	**:**	Evaluation of association panel for machine harvest traits in lentil
Objectives	**:**	Phenotype the association panel for machine harvest traits
Activities	**:**	New
Expected outcomes	**:**	Marker-trait associations, potential QTL, and beneficial alleles for machine harvest traits would be identified.
Observations to be taken	**:**	Plant height Height of the lowest pod Phenological traits Pod drop Pod dehiscence Canopy height Vine length Plant Height Index Yield and yield components
Genotypes	**:**	ICARDA GCP core – collection and heat and drought lines.
Key outcomes	**:**	• GBS using the two-enzyme (*Pst*I, *Msp*I) method was deployed on 185 lentil accessions including ICARDA lentil GCP- reference collection lines as well as 14 abiotic stress responsive lines derived from diverse environments. • The raw sequence data was processed to remove low quality data and analyzed using the Stacks software package, version 1.19 and in-house scripts were used to call 22,029 high confident SNPs. • Sequences were used as queries in a BLASTX search (version 2.2.24), and the sequence alignment was done for each sequence. It was run against the non-redundant database with an e-value of < 1x E-6. • Species distribution chart revealed the first four matches from two cool season legumes *Medicago truncatula* and chickpea, followed by the warm season legumes soybean and common bean (Figure 1).

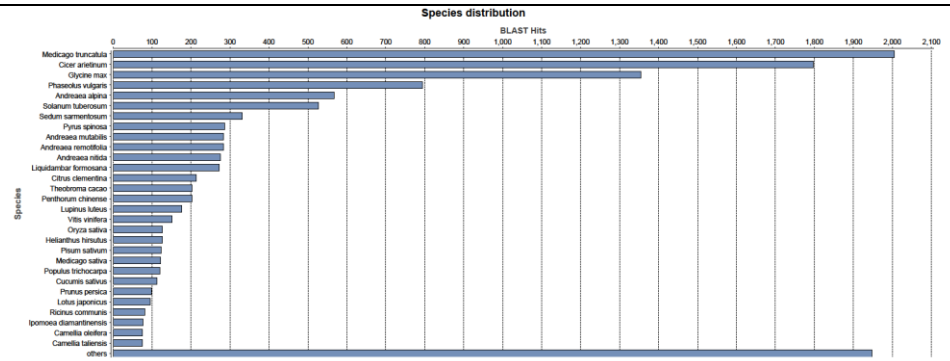


Figure 1. Species distribution chart of lentil gene chip after Blastx to NCBI nr. The four highest BLAST matches were to two cool season legumes *Medicago truncatula* and chickpea, followed by the warm season legumes soybean and common bean.

- About, 10,277 (46.65%) SNPs mapped to the reference genome of *Medicago truncatula* version 4.1 (minimum E-value < 1.0 e-10 and 80% identity) using BLASTN, indicating high levels of conserved synteny of both species (Figure 2).

Figure 2. 10,277 (46.65%) SNPs mapped to the reference genome of *Medicago truncatula* version 4.1 (minimum E-value < 1.0 e-10 and 80% identity) using BLASTN. Below is a partial view of *M. truncatula* chromosome 1 and matched lentil SNPs. The lentil SNP data is available as a track on the Cool Season Food Legume Database Medicago Gbrowse (www.coolseasonfoodlegume.org).

- InterPro domains were assigned and associated gene ontology (GO) terms describing the genic SNPs were determined using BLAST2GO and in-house scripts.
- Eight marker-trait associations were identified, suggesting the usefulness of GWAS approach (Table1).

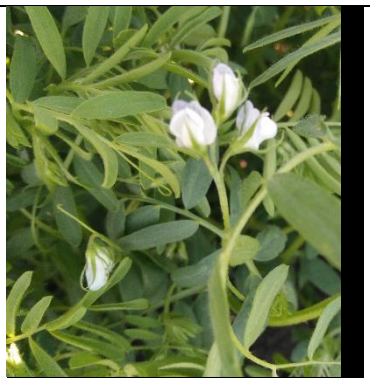
Table 1. The preliminary results of GWAS describing the marker-trait associations in agronomic traits.

Trait	**Marker**	***P***	r^2
Seed weight	986	0.0002	0.10
Seed weight	9594	0.0005	0.10
Seed weight	5617	0.0007	0.09

			Plant height	13524	0.0008	0.10	
			Days maturity	824	0.0001	0.13	
			Days maturity	244	0.0007	0.13	
			Seeds per pod	10700	0.0008	0.07	

Activity 3: Application of TiLLING approach for lentil crop improvement.

Title	**:**	A study of TiLLING approach to elucidate gene function in a gamma irradiated lentil mutant population
Objectives	**:**	Identify novel mutants for the introduction of novel allelic variation in lentil breeding
Activities	**:**	New
Expected outcomes	**:**	TiLLING platform of lentil developed. Novel mutants with various beneficial economic traits would be identified.
Observations to be taken	**:**	Morphological traits Phenological traits Yield and yield components
Varieties	**:**	ILL4605 and ILL5883
Key outcome	**:**	Lentil mutant populations were generated for two varieties namely ILL4605 and ILL5883 with three doses of gamma rays including 50Gy, 100Gy and 150Gy. A total of 2774 single plant selections 6140 SSD were made at M1 generation of ILL 4605. For ILL5883, 285 single plant selections and 8758 SSD were made at M_1 generation of ILL5883. Interesting mutants were identified. (Plate1). A  B 

		C  D  E  F  **Plate 1.** Chlorophyll mutants and mutants (A, B) with three flowers per peduncle (C,D) and stunted growth (E) and large tendrils (F) identified in ILL 4605 M_1 population.

Activity 4: Generation advancement of lentil RIL population.

Title	**:**	Generation advancement of lentil RIL population
Objectives	**:**	Forward RIL population to next generation
Activities	**:**	Continued
Expected outcomes	**:**	RIL population at advanced stages would be used for linkage mapping and phenotyping in multiple locations.
Observations to be taken	**:**	Phenological traits
Populations	**:**	A total of 30 RIL population at F_2 generation forwarded to next generation in Marchouch and 33 RIL population at F_3, F_4 and F_5 generation is advanced for next generation in Terbol.

Key outcome	**:**	All population are advanced for one more generation through SSD method.

Activity 5: Nine Mapping of Fusarium wilt resistance gene in chickpea

Title	**:**	Screening of the chickpea genotypes and breeding lines for Fusarium wilt resistance in chickpea
Objectives	**:**	Identification of a new gene related to furarium wilt resistance in chickpea
Activities	**:**	Continued
Expected outcomes	**:**	New gene conferring resistant to Fusarium wilt in chickpea
Observations to be taken	**:**	Wilted plant Score %
Genotypes	**:**	150 RILs derived from cross between FLIP 97-7 and ILC482

Results:

A total of 23 SSR markers have been linked and associated with FW resistant gene identified on chromosome 5. A total of 9 RILs showed heterozygous SSR pattern at the QTL region. A total of 137 plants derived from these lines were planted in the field to confirm their resistance/susceptibility. The DNA of these plants was also extracted for further fine mapping. The primarily phenotyping confirmed the segregation of the resistance within the RILs. Genotyping and data analysis is still in progress to identify the resistant gene in FLIP97-7.

Activity 5: Development of a new F2 population conferring Glyphosate tolerance in chickpea

Title	**:**	Development of a new F2 population conferring Glyphosate tolerance in chickpea
Objectives	**:**	Identify marker linked to Glyphosate tolerance in chickpea
Activities	**:**	New
Expected outcomes	**:**	Markers linked to Glyphosate tolerance RIL population will be developed later for mapping and QTL analysis
Observations to be taken	**:**	Toxic symptoms on the F2 individuals
Genotypes	**:**	191 F2 plants derived from cross X014TR-16 (FLIP08-115XFLIP07-268)
Results	**:**	A total of 191 F2 population were evaluated in Terbol station, Lebanon by using Glyfosate360g herbicide. The primary results indicated that the tolerance was dominant, but the segregation of the tolerant gene(s) did not fit 3:1 (the Mendelian ratio) indicating the possibility of more than single dominant gene control the Glyphosate tolerance in chickpea.

The DNA from each F2 plant was collected and stored at -20 C for further molecular study.

	Observed	Expected
Tolerant	160	143.2
Sensitive	31	47.8
Chi squared = 4.513; P=0.0336		

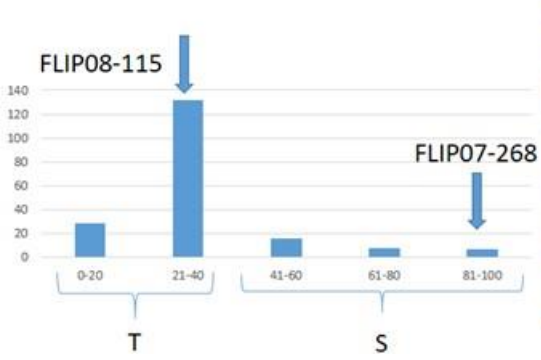


Figure 1: Number of F2 plants grouped into different categories based on the % of the toxic symptoms. T: tolerant genotypes (0-40%); S: susceptible genotypes (41-100%).

Activity 7: Study of F_2, F_3, F_5 and F_7 progenies for yield, mechanical harvestable traits and market class related traits in Faba bean

Title	**:**	Evaluate the single plant progenies for yield and desirable morphological traits
Objectives	**:**	Evaluate the single plant progenies at F_4 and F_5 and forward the superior progenies to next generation.
Activities	**:**	New
Expected Outcomes	**:**	High yielding varieties with beneficial traits including extra earliness, machine harvesting would be developed.
Observations to be taken	**:**	Phenological traits Yield and yield components Seed size and seed color classification
Crosses and progenies	**:**	At Terbol, the following generation were planted • 1917 F3 single progenies from 35 crosses and 1660 F4 single progenies from 40 crosses were forwarded for evaluation in single row plot in open field condition. Those lines were selected for earliness, photoperiod insensitivity and for thermotolerance. • F3 bulk population designed for earliness and for heat tolerance was planted at Terbol in isolation and in open field. • 285 F5 progenies, 415 F6 progenies were planted under screen houses for evaluation for mechanical harvestable traits and earliness and for seed purification • 20 Synthetic populations developed and forwarded to Syn2 generation at Terbol station At Kafardan the following bulk segregating population were planted: • 240 F2 populations covering the following traits: drought, orobanche and disease resistance, heat, glyphosate resistance, low tannin population and mechanical harvestable lines. these population with evaluated for earliness, and other agronomical traits • 116 F3 bulk population developed for disease resistance, orobanche resistance, heat tolerance large seeds and extra early flowering
Results		• 2500 F4 and 1500 F5 SPS identified for earliness and mechanical harvestable traits were identified from the above populations. Among them 1200 F4 and 500 F5 will be screened for chocolate spot and rust in coastal are next season. 1300 and 1000 were planted in offseason 2016 and will be screened for earliness and heat tolerance

		• 700 single plants were selected for their agronomic performance (number of pods/plant), and mechanical harvestable traits. Those lines will be screened for orobanche resistance next season in Sids station, Egypt. • 1300 F3 and 750 F4 single progenies were selected from population obtained by crossing Heat tolerant sources and forwarded to summer season 2016 • The 20 Synthetics developed yielded between 3 to 4 tonnes per ha, The produced seeds will be sent to partners though international nurseries 2018 • The F2 and F3 populations planted under rainfed conditions in Kafardan were exposed to drought with total rainfall of 250 mm. selection was made under this condition in F3 bulk population and the F2 populations were harvested and forwarded to F3 generation. • 70 F3 populations obtained from crosses of large seeds and disease resistance with Indian germplasm and with sources for heat tolerance were planted under insect proof cages during summer season 2016. • 200 F5 lines selected previously for heat, low tannin, large seeds were purified under insect proof cages and forwarded to PSN nurseries

Activity 8 : Development of faba bean MAGIC population

Title	**:**	
Objectives	**:**	Development of faba bean magic population for resistance to orobanche resistance chocolate spot, asoschyta blight and rust resistance and heat and drought tolerance/
Activities	**:**	Continue
Observations to be taken	**:**	Flowering time, plant height, pod number, seed number
Crosses and progenies	**:**	8 parents identified for development of magic population. 28 crosses under development
Results	**:**	560 single F1 magic population developed. The 8 parents lines used for magic population were evaluated for 794 KASAP markers and showed enough diversity to be selected as parent for the development of the magic populations.

Activity 9: Development of faba bean TILLING population

Title	**:**	Mutagenized M1 population
Objectives	**:**	Development of faba bean tilling population for further screening
Activities	**:**	New

Activity		On going
Observations to be taken	**:**	phenology
Results	**:**	M1 tilling population obtained from mutagenizing NA112 lines by EMS. 1200 M1 single plants obtained The M1 plants were harvested and it will be advanced to next generation.