RESEARCH PROGRAM ON Livestock

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Science for resilient livelihoods in dry areas

Technical Progress Report
Unlocking the Potential of Grass pea for Resilient Agriculture in Dry Environments (UPGRADE)

Test performance of low and high ODAP genotypes in field and controlled conditions

Reporting Period: June 2019- Dec. 2021

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ICARDA in partnership with the John Innes Centre and the Ethiopian Institute of Agricultural Research has carried out various experiments envisaged in the work packages of the project entitled **"Unlocking the Potential of Grass pea for Resilient Agriculture in Dry Environments (UPGRADE)".** This report covers activities implemented by ICARDA under WP1 and WP2 during the period June 2019 – Dec. 2021.

WP2: Test performance of low and high ODAP genotypes in field and controlled conditions

a) Yield performance of low ODAP grass pea in fields

During 2020, the project team evaluated 25 grass pea lines for their performance at two locations (Terbol and Marchouch). These low ODAP lines emanating from various crosses showed 0.27 to 28.21% yield advantage over the local check (Table 4). ODAP concentration of these lines is being analysed.

Table 4. Yield performance of grass pea lines in Marchouch, Morocco

Entry	Pedigree	Designation	Grain yield Kg/Ha			Yield advantage over the check %
			Terbol	Marchouch	Mean	
18	IGC-2011-32	IGC-2011-32-605	2309.00	3488.89	2898.94	28.21
3	IGC-2011-7	IGC-2011-7-121	2220.00	3166.67	2693.33	19.12
21	IGC-2011-40	IGC-2011-40-709	2174.00	3192.59	2683.30	18.67
17	IGC-2011-31	IGC-2011-31-591	2272.00	3048.15	2660.07	17.65
15	IGC-2011-31	IGC-2011-31-586	2702.00	2553.70	2627.85	16.22
5	IGC-2011-16	IGC-2011-16-278	2313.00	2720.33	2516.67	11.30
6	IGC-2011-16	IGC-2011-16-281	2006.00	2990.74	2498.37	10.49
7	IGC-2011-18	IGC-2011-18-327	2088.00	2905.56	2496.78	10.42
2	IGC-2011-5	IGC-2011-5-67	1992.00	2918.52	2455.26	8.59
12	IGC-2011-27	IGC-2011-27-509	1648.00	3240.74	2444.37	8.11
20	IGC-2011-34	IGC-2011-34-646	1846.00	3027.78	2436.89	7.77
19	IGC-2011-33	IGC-2011-33-630	2181.00	2687.04	2434.02	7.65
14	IGC-2011-30	IGC-2011-30-561	2201.00	2657.41	2429.20	7.43
33	IGC-2011-62	IGC-2011-62-1114	1619.00	3207.41	2413.20	6.73
35	IGC-2011-86	IGC-2011-86-1402	2110.00	2701.85	2405.93	6.41
8	IGC-2011-18	IGC-2011-18-335	1752.00	2998.15	2375.07	5.04
10	IGC-2011-21	IGC-2011-21-387	1776.00	2929.63	2352.81	4.06
22	IGC-2011-40	IGC-2011-40-712	1819.00	2879.63	2349.31	3.90
13	IGC-2011-29	IGC-2011-29-547	1747.00	2914.81	2330.91	3.09
1	IGC-2011-5	IGC-2011-5-64	1867.00	2672.22	2269.61	0.38
23	IGC-2011-41	IGC-2011-41-738	2166.00	2368.52	2267.26	0.27

Trial mean			1747.94	2779.32	2263.63	
Trial Max			2702.00	3488.89	2898.94	
Trial Min			813.00	2274.07	1650.02	

b. Screening of grass pea germplasm for various abiotic stresses and their effect on ODAP concentration

From November 2020, the project team hired a consultant, Dr. Priyanka Gupta to carry out screening of grass pea germplasm against drought, waterlogging, salinity, and heat stress. For these experiments, 64 diverse genotypes were selected based on phenology (early/late flowering), sensitivity to Orobanche, and ODAP content. Various experiments carried out and their status are presented below:

Screening against water stress: Root system plays an essential role in drought tolerance and therefore, the project team evaluated root trait characteristics (root length, root density, and root biomass) and root system architecture (RSA) of 64 grass pea genotypes by deploying the following four methods: Non-paper roll, Clear pot, Rhizotron, and Basket (Pasta strainer) method (Fig 1). While the first three methods provided information on root traits at seedling stage, the basket method provided information not only on root traits but also on the performance regarding phenology, grain and biological yield and ODAP content.

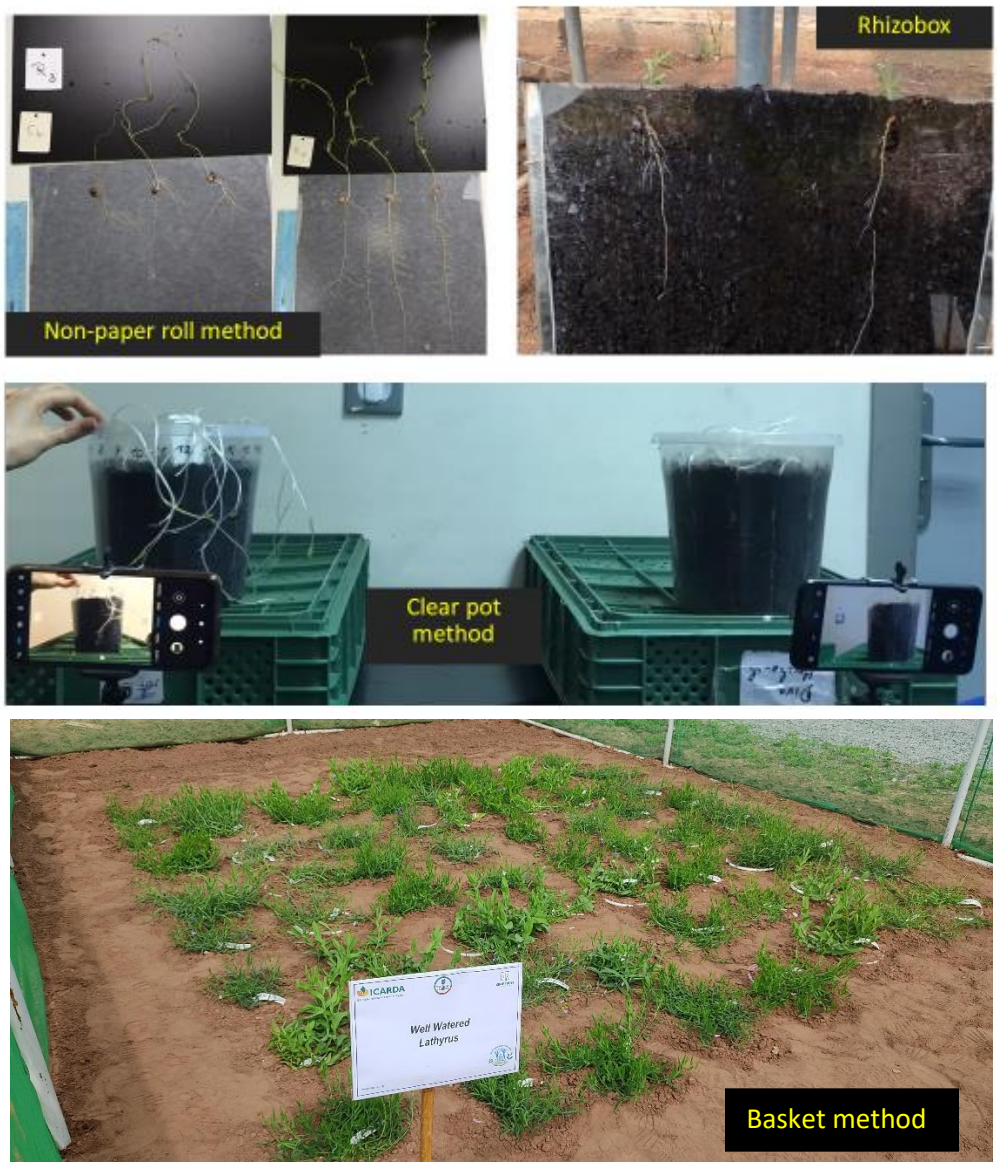

Figure 1. Different methods for assessing root traits of grass pea germplasm: (i)Non-paper roll method (top left); (ii) Rhizobox (top right); (iii) Clear pot method (middle); and (iv) Basket method (bottom)

a) Non-paper roll method: 64 diverse grasspea germplasm were characterized for RSA traits at the seeding stage using the protocol of Canè et al. (2014). Briefly, seeds were manually selected for uniformity, weighed, sterilized in a 1% sodium hypochlorite solution for 10 ′, rinsed in distilled water, and germinated in Petri dishes at 28°C for 24 h. Homogeneously sprouting seedlings were then grown in moist filter paper sheets in vertical black polycarbonate screening plates (40×35 cm). Seedlings were grown for 28 days at 22 hours photoperiod and under a controlled temperature around 20 ±2°C (day and night) using

LED-based lighting regimes. Light-emitting diodes (LEDs) systems allow very specific adjustments regarding light intensity and quality. The results showed significant variability for root traits at seedling stage. Two genotypes, namely DR20 (*L. sativus)* and IG65252 (*L. cicera*) had the deepest root system along with high number of total roots count and weight while two genotypes IG 64810 and IG 64813 (*L. ochrus*) had most widely distributed root system with highest TRN count (Fig. 2). Statistical analysis is underway.

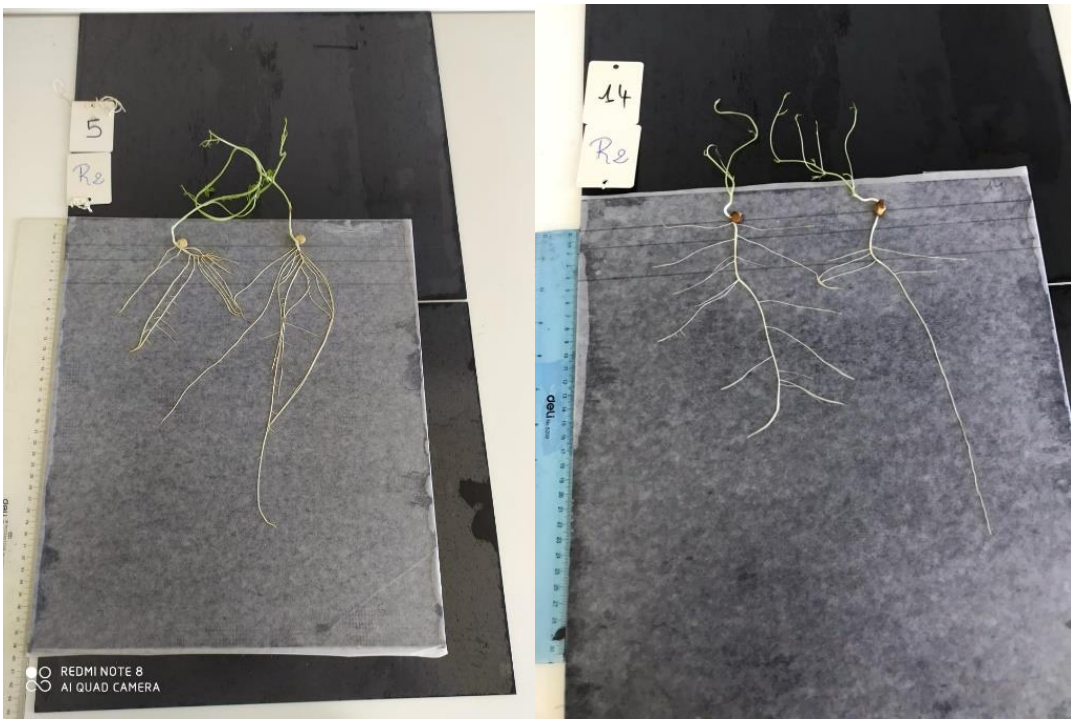

Figure 2. Root trait variation in grass pea genotypes at seedling stage using non-paper roll method

b) Clear pot method: The clear pot method allows high-throughput and cost-effective screening of grass pea germplasm. The project team used 50 transparent pots (top diameter:20cm height-19cm) filled with 100% peat mass soil. Sixty-four diverse grass pea genotypes with 12 replications were planted in clear pots using RCBD design. Twelve seeds per pot were planted close to the pot wall with embryo facing downward at ~3-cm depth (Richard et al. 2015) using forceps. Images were captured for each plant after 16 days using digital camera fixed on a tripod with antireflection walls. Seminal root angle (SRA) estimated using ImageJ software (http://imagej.nih.gov/ij/). The project team did not measure any other root characteristics like primary root length and total root number as

roots were hidden by the peat mass soil. However, shoot length and shoot nodes were recorded. Three best SRA/seed emerging from primary root were analysed on ImageJ. Data analysis is in progress for genetic variation of root traits of grass pea genotypes.

Figure 3. Root traits observations of different grass pea genotypes following clear pot method

c) Rhizotron: To study root system behaviour and plasticity, rhizoboxes were used because they allowed continuous observation of uninterrupted root growth compared to plant cultivation by other means, e.g., pots or in the field, which only allow samples to be taken, hence disturbing or killing the individual plants under study. These rhizoboxes were made of plexiglass sheets (poly-methyl methacrylate (length: 48cm, Width: 37.5 cm and 3.5cm space between two transparent glass). The assembly was held together by special glue stick through two aluminium-channels on the sides, and holes were formed close to the bottom rhizotrons for proper drainage and aeration. After preparation, the soil mix (with 70% peat mass) were manually spread uniformly, then compressed to ensure that the surface is level with the glass silicon strips. After adding the glass plates, the system is closed as described above. Two seeds per box were planted at 2cm below from upper surface. Rhizotron were kept at an angle of 45° by metallic supports in plastic house at Rabat. Currently the experiment is underway where genotypes are showing clear difference in RSA root pattern (Fig. 4).

Figure 4. Root traits variation for grass pea genotypes under rhizotron experiment

d) Pasta strainer (basket) method:

The same set of 64 grass pea genotypes were planted at ICARDA-INRA station, Rabat, Morocco on December 28, 2020. For phenotyping root architecture, open plastic mesh baskets (top diameter of 29 cm, bottom diameter of 21.5 cm, height of 9.5 cm, and mesh size of 3-5 mm) were used (Uga et al 2009). These baskets were filled with soil mixed evenly with fertilizer @ 15N:15P:15K before planting. These baskets were buried in the field maintaining a spacing of 50 x 25 cm between baskets. The experimental design was alpha lattice with two replications and two treatments, well-watered (WW) and water stressed (WS) (Fig. 5). Observations will be recorded for above ground traits like germination, days of heading, plant height, shoot nodes, total pods number, seed number,100-seeds weight, seed weight and total biomass weight. For underground observation at maturity, the baskets were pulled out carefully and soil will be removed around the basket from the field. To understand the root growth pattern, root growth angel (RGA) will be recorded at three levels of basket and then washed. Further, to study the root physiology, images of root lengths will be taken following WinRhizo and ImageJ software as well as other traits like root level per ratio and root biomass. Seeds of each grass pea genotype harvested under well-watered and water stressed conditions will be analysed for ODAP content to assess the effect of drought on ODAP content of high and low ODAP grass pea.

Figure 5. Root trait assessment of grass pea genotypes using basket method

Screening against waterlogging: Grass pea crop encounters waterlogging situation at different growth stages. Exposure to extended period of waterlogging may cause membrane deterioration and leakage of cellular contents, resulting in germination failure and/or seed death and further growth (Zaman et al., 2019). Keeping this in mind, two experiments were conducted to evaluate waterlogging effect on grass pea growth. The first experiment was carried out at seedling stage in small germination trays and another one allowed until maturity in cones under plastic container. In both experiments, trays and cones were filled with soil and peat moss at 70:30 ratio and provided water for three days at 100% field capacity before sowing. Planting was done on March 25, 2021 in glasshouse. Two seeds of each of 64 genotypes were planted in a set of 24 spaced tray (6 X 6 cm) following RCBD design with two replications. Two factors were included: First factor was duration of waterlogging (no waterlogging and 10-day waterlogging). Waterlogging was maintained at 10 mm above surface for 10 days followed by 15 days of drainage. These trays were kept in the plastic container having 56L water holding capacity. The second experiment included the same 64 genotypes where seeds were germinated in cones for a week followed by 21-day waterlogging and then the water was drained out for 15 days for recovery. A 1 to 5 scale was used to record the reaction of sensitivity of genotypes against waterlogging (0 = completely dead plant, 1 = 75-99% wilt, 2 = 50-75%, 3 = 25-50%, 4 = <10% and 5: full green and healthy plant. Three genotypes namely IG115437, IG64798 and IG64183 germinated successfully under waterlogging (Fig. 6). These genotypes reflecting quiescence mechanism which could germinate from the first day of waterlogged treatment. The experiments are still in progress and data are being recorded.

Figure 6. Screening grass pea germplasm against waterlogging

Screening grass pea against heat and combined heat + drought: The purpose of this experiment was to evaluate 64 grass pea under heat stress at reproductive and combined effect of heat + drought stress to assess the effect of heat and drought on ODAP concentration. For this, 64 genotypes were planted in a randomized complete block design (RCBD) with two replications on 23 Feb 2021 at plastic house, Rabat, Morocco. All pots were watered at 80% field capacity before sowing followed by irrigation as per requirement till flowering. Three treatments, control, heat and heat+ drought was examined under two plastic houses. One plastic house kept at control condition temperature (20 to 25^{0}C) and another was set on up to 35^{0}C. Data loggers were placed at both the plastic houses to record hourly temperature and humidity. In case of control and heat treatments, water was given twice a week to maintain 70% field capacity whereas water stress was imposed after first flowering in the combined treatment of Heat + Drought. This experiment is under progress and agronomic and physiological data are being recorded. Experiment is expected to complete by July (**Fig. 7**).

Figure 7. Screening of grass pea germplasm against heat and combined heat + drought stress

Screening grass pea against salinity: A hydroponic experiment was conducted to examine 26 grass pea genotypes for their reaction against salinity. Seeds were germinated under sterile condition and similar size seedling were transferred to containers. Each container filled with 30 L Hoagland nutrient solution with constant aeration. Six hydroponic containers each with 26 slots were used in RCBD design along with two replications for three treatments (control, 50 mM NaCl, 100mM NaCl). Solution was checked and refilled every four to six days depending on the requirement and pH was monitored in the range of 6 to 6.5. After 4-5 weeks, observations were recorded on root length, shoot length, root dry weight, aboveground biomass dry weight and aboveground biomass fresh weight. The tolerant lines will be checked for Na and Ca concentrations. The experiment is underway to assess their tolerance against salinity.

Figure 8. Screening grass pea germplasm against salinity stress

The UPGRADE activities under WP 1 and 2 are progressing very well where data were collected on performance of grass pea both under abiotic and biotic stresses. The analysis of various pot and plot experiments and laboratory analysis of ODAP content will provide the opportunities to develop grass pea lines with low ODAP content. Some of these results will be reported in the next report.

***Consent Statement**: "Personal information including Name, Business title, Email, Phones, Images and GPS points included in this report have been authorized in writing or verbally by the data subject" Z. Bishaw*