Report of a *Lactuca aculeata* collecting expedition in Jordan

Itinerary, collected material and data

Kik C, K Abulaila & Z Tahabsom

WAGENINGEN
UNIVERSITY & RESEARCH

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Centre for Genetic Resources, the Netherlands (CGN), Wageningen University & Research
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CGN is ISO 9001 certified.

Picture front page: *Lactuca aculeat*a in a farmer's field with barley (ATK 035).

Contents

Preface

The mission of the Centre for Genetic Resources, the Netherlands (CGN) is to contribute to the conservation, development and sustainable use of plant, animal and forest genetic resources, and hence to global food security, a more sustainable production, rural development, and the conservation of cultural heritage.
To that end, CGN currently holds collections of over 20 crops and a total number of accessions of more than 22,500 of interest to the breeders, researchers and other users. Annually around 5000 seed samples are distributed.

To contribute to an effective global system of *ex situ* collections, for each of its collections CGN has analysed the coverage of the crop genepool by the germplasm in its own collection and those of others. In a number of cases, CGN has been able to identify gaps in the total set of collections of a specific crop. Some genetic diversity that is known or can be assumed to exist, appeared poorly represented or even absent from the genebank collections. Such cases warrant new collecting missions, if we wish to conserve as wide a diversity for the crop genepool as possible.

The species *Lactuca aculeata* forms such a case. Therefore in 2017 CGN carried out a collecting mission in Jordan, in close collaboration with its local counterparts. The present report provides details of the results of this collecting mission. Five plant breeding companies co-financed the mission, a fact that is duly recognized and appreciated.

During the mission fifty-five seed samples were collected. Upon regeneration, the samples will be made available under the terms and conditions of a Material Transfer Agreement, with the agreement of the authorities in Jordan involved.

This collecting mission formed an activity jointly undertaken by partners in Jordan and the Netherlands. The support from the national authorities in Jordan is duly recognized.

1 Introduction

In the global plant genetic database GENESYS (www.genesys-pgr.org), 15,258 *Lactuca* accessions are present amongst which 12,129 *Lactuca sativa* and 2946 crop wild relatives (CWR). *Lactuca aculeata* (Boiss. & Kotschy) is a rare species in the GENESYS database with only 23 accessions present of which 15 originate from Israel and 1 from Turkey. Seven genebanks maintain these accessions with the Israeli genebank maintaining most accessions, namely 11. The Dutch national genebank, CGN, maintains only two accessions, whereas the genebank at NCARE in Jordan maintains no accessions of this species. As *Lactuca aculeata* belongs to the primary genepool of lettuce (*Lactuca sativa*; Globerson *et al.* 1980; Zohary 1991), it is a relatively easily exploited gene reservoir for the breeding of new lettuce varieties. In this context the presence of resistance against several physio's of *Bremia lactucae* (Beharav *et al.* 2006, 2010a, Jemelkova *et al.* 2015) is of considerable practical interest for lettuce breeders. Furthermore the species has been analysed in fundamental studies on *Lactuca* phylogeny (Koopman *et al.* 1998, 2001), population genetics (Kitner *et al.* 2015), and sesquiterpene lactones phytochemistry (Beharav *et al.* 2010b, Michalska & Kisiel 2012). The known distribution area of *Lactuca aculeata* is presented in Figure 1 (see for further details: Zohary 1991, Jemelkova *et al.* 2015).

Figure 1 *Distribution area of Lactuca aculeata.*

Given the fact that only a few accessions of *Lactuca aculeata* are present in genebanks worldwide and that the species could harbour agronomically interesting traits, a collecting mission to Jordan, a country that is located within the centre of biodiversity of the species, is clearly warranted. Therefore in the context of an already existing joint plant genetic resources project

between NCARE (Jordan) and CGN (the Netherlands), a collecting project was set-up to sample *Lactuca aculeata* from Jordan.

In order to make such a collecting mission possible a Memorandum of Understanding using a tailor-made Material Transfer Agreement (MTA) as a basis for distribution was signed in the first half of 2017 between the appropriate authorities of both countries and this document formed the legal basis of the expedition (Appendix 1).

2 Objectives of the expedition

The major aim of this single crop expedition is to broaden the *Lactuca aculeata* collections maintained at CGN and NCARE by collecting wild populations which can subsequently be used after regeneration for breeding and research purposes, which consequently contributes to the international need for the conservation of plant genetic resources (PGR).

3 Members of the collecting team

- Khaled Abulaila and Ziad Tahabsom, National Centre For Agricultural Research And Extension (NCARE), Baqa', Jordan; E-mail: kabulaila@gmail.com and ziad1000@yahoo.com
- Chris Kik, Centre for Genetic Resources, the Netherlands (CGN), Droevendaalsesteeg 1, 6708 PB Wageningen, the Netherlands; E-mail: chris.kik@wur.nl

4 Exploration area and expedition period

The exploration area was situated in the western part of Jordan. The area covered during the mission was from North to South Jordan ca. 350 km and from East to West ca. 50 km. In total around 3000 km was travelled during a

period of three weeks (September 10 – October 1). Most populations were found North of Amman (Figure 2).

Figure 2 *The exploration area in Jordan with the locations of the various Lactuca accessions indicated (see for details Appendix 3).*

Temperatures during daytime in Jordan were in the first half of the expedition between 35-40 ˚C and in the second half between 25-30 ˚C. For transport a Ford ranger was used. Most of the expedition was carried out using Amman as a basis. Only in the last part of the expedition, which took place in South Jordan, hotels were used.

5 Data collecting, sampling procedure and seed cleaning

A field collecting form based upon a modified multi-crop passport descriptor list (MCPD; see: http://eurisco.ipk-gatersleben.de) was used to document the passport data of the accessions sampled (Appendix 3). All sampled material received a so-called collecting number, in this case ATKxxx. Latitude, longitude and altitude were determined via GPS (Garmin, eTrex 20) with an inaccuracy of 1-5 meters. Latitude and longitude were recorded using as map datum WGS84 and as position format hddd°ddddd. Pictures were taken of the collecting site. Passport data can be found in Appendix 4.

Sampling of all *Lactuca* species, except *Lactuca orientalis*, took place on individual plants and the seeds of minimum two-three flowering heads were

collected per individual plant. If not more than two-three flowering heads could be collected these seeds were transferred to one (= single) glassine bag. If a minimum of four-six flowering heads with ripened seeds were present per individual than two seed (= double) glassine bags per plant were collected (see Appendix 4). These two glassine bags were stapled to each other. Subsequently the single and double bags per accessions were put together in one cotton bag (18 x 30 cm^2) which was bind together with a string. A plasticized paper tag with the collecting number was placed on the inside and on outside with a string which tied together the cotton bag. The glassine bags were individually marked during the expedition to be able later on to trace the origin of the seeds within a population. This was done by writing on the glassine bags the accession number, the number of the individual and an 'a' or a 'b' in case the seed of an individual was transferred into two bags (example: ATK032-01-a or ATK032-01-b in case of seed from population ATK032 plant number 1 was harvested into two glassine bags and ATK033-01 in case the seed of plant number 1 of population ATK033 was harvested into one single glassine bag). During the expedition the cotton bags (with seeds) were kept under ambient room conditions. Cleaning of the seeds during the expedition was not necessary as the seeds were already reasonably clean when harvested. Upon arrival at CGN the seeds were transferred to a conditioned storage room with a temperature of 15°C and 15% relative humidity and a receipt number (RNR) was assigned to each accession.

The *Lactuca orientalis* population was collected as a bulk because from individual plants it was difficult to obtain enough seeds.

6 Results and discussion

In total 55 populations were collected: 51 *Lactuca aculeata*, 1 *Lactuca serriola* (ATK024) 1 *Lactuca saligna* (ATK031), 1 *Lactuca serriola/saligna* (ATK022) and 1 *Lactuca orientalis* (ATK055). Furthermore a *Lactuca undulata* (ATK-un) population was identified which could not be sampled as the plants were already withered (see Appendix 4).

6.1 Lactuca aculeata

The overriding majority of the *Lactuca aculeata* populations were discovered from around 20 km south of Amman to the North. Further south of Amman the desert begins, a habitat in which no *Lactuca aculeata* was found. The habitat in which *Lactuca aculeata* grows is a disturbed habitat (Photo 1a,b,c). Mostly *Lactuca aculeata* was found on roadsides and soil waste deposits along roads (n=31), but also in olive orchards (n=6) and in barley (n=2) and grape fields (n=1).


a


b

Photo 1 a,b,c *Lactuca aculeata collecting sites: a: road from Amman to Queen Ali airport (ATK032), b. a barley field in the neighbourhood of Huwara (ATK035) and c. in a vineyard close to Aljun castle (ATK026).*

The average elevation where *Lactuca aculeata* grew ranged from 513-1438 m with an average of 812 m, which was slightly higher than observed in Israel where the species grew in between 222-968 meters (Beharav *et al.* 2010).

The habitat preference of *Lactuca aculeata* and another *Lactuca* species, namely *Lactuca serriola*, which also occurs in disturbed habitats, was not clear. If *Lactuca aculeata* was present, then also *Lactuca serriola* was present, but vice versa was not the case. However what the difference was of the habitats in which only *Lactuca serriola* occurred versus the habitats in which both *Lactuca* species occurred, did not become clear during the mission. As both species grew often together in one location, the presence of hybrids could be expected as has been shown by Lebeda *et al.* (2012). No obvious case was encountered during the mission where the presence of hybrids between both species was clear. Perhaps this is due to the fact that hybrids morphologically resembled to a large extent *Lactuca serriola* (see Lebeda *et al.* 2012) and that only via marker analysis the presence of hybrids can be confirmed.

The *Lactuca aculeata* populations sampled varied dramatically in the estimated number of individuals present, namely from 1 to larger than 100 (Table 1). Eight populations were even larger than 500 individuals (Appendix 4).

Table 1 *The number of Lactuca populations per size class; class width: a<x≤b; except for class 1-10 where the width is defined as a ≤x≤b.*

category	1-10	10-100	>100
Lactuca aculeata	14	19	18
other *Lactuca* species		1	3

The total number of plants from which seed was sampled during the expedition was 1370 (for *Lactuca aculeata* this number was 1285 and for the other *Lactuca* species 85). The ranges, means and their standard errors of the number of plants, the number of single bags and the number of double bags per *Lactuca aculeata* population from which seed was sampled is presented in Table 2.

Table 2 *The ranges, means and their standard errors of the number of plants harvested, the number of single glassine bags and the number of double glassine bags per Lactuca aculeata population from which seed was harvested.*

	range	mean	standard error
number of plants	1-67	25	21
single bags	0-44	12	13
double bags	0-67	13	15

From Table 2 it becomes clear that populations differed to a large extent (range 1-67) from each other with respect to the number of plants from which seed could be harvested. On average 25 plants per population were harvested and from these seeds of 12 plants were transferred in single glassine bags and seeds from 13 plants were put in double glassine bags.

6.2 Other *Lactuca* species

Most *Lactuca serriola* populations encountered had already shed their seed, but in case of ATK 024 it was still possible to harvest seeds. *Lactuca saligna* was rarely encountered, however it was found at a highway merger south of Amman (ATK031) where also a very large population of *Lactuca aculeata* was present. No visible hybrids between both species were observed. Putative hybrids between *Lactuca serriola* and *Lactuca saligna* were seen at the ATK022 location in the village of Ebbein. The inflorescence of the plants in this population resembled *Lactuca serriola* whereas the leaves resembled *Lactuca saligna*.

Photo 2 *Lactuca saligna population (ATK031) which was sampled.*

Lactuca undulata (ATK-un) and *Lactuca orientalis* (ATK055) are true desert species (Photo 3a,b,c). Both species existed of small size individuals. It was not possible anymore to sample the (only?!) *Lactuca undulata* population in Jordan as all plants were already withered and had shed their seed. For *Lactuca orientalis* the situation was differently as flowering and seed carrying plants were found. Sampling the seed from plants was difficult as they were small and extremely spiny, therefore a bulk sample was taken.

Photo 3a,b,c *Habitats of desert Lactuca species: a. habitat of Lactuca undulata, b. habitat of Lactuca orientalis and c. a flowering and seed bearing Lactuca orientalis plant.*

7 Conclusions

a. This collecting mission was carried out in the context of a joint PGR project between NCARE (Jordan) and CGN (the Netherlands). A MTA was signed between the competent national authorities of Jordan and the Netherlands. This formed the legal basis of the expedition.
b. Fifty-five *Lactuca* accessions were collected during the mission which can be subdivided in 51 *Lactuca aculeata*, one *Lactuca serriola*, one Lactuca saligna, one mix population of *Lactuca serriola* and *Lactuca saligna* and one *Lactuca orientalis*.
c. Seed samples were taken from individual plants; only the accession of *Lactuca orientalis* was sampled as a bulk.
d. In total from 1370 plants seeds were harvested in glassine bags; from *Lactuca aculeata* 1285 plants were sampled.
e. For *Lactuca aculeata* in between 1 and 67 plants seeds were harvested per population with an average of 25 per population.
f. No clear cases of species hybridization in which *Lactuca aculeata* was one of the parents could be established. A population where possibly hybridization occurred between *Lactuca* serriola and *Lactuca saligna* was identified.
g. *Lactuca undulata* could not be sampled as the plants were already withered and had shed their seeds.

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9 Acknowledgements

This expedition was made possible by the financial support from breeding companies affiliated to PLANTUM-NL and the Dutch Ministry of Economic Affairs. The support and hospitality of local people in Jordan was of great value. Furthermore CK would like to express his sincere gratitude to the Khaled Abulaila and Zaid Tahabsom who made this collecting mission a success. For the critical reviewing of this report Rob van Treuren is greatly acknowledged.

Appendix 1 MTA

MATERIAL TRANSFER AGREEMENT

PREAMBLE

In reference to the Jordan-Netherlands collaboration regarding safeguarding and use of plant genetic resources native to Jordan, and the Memorandum of Understanding signed between the National Centre for Agricultural Research and Extension (NCARE) and the Centre for Genetic Resources, the Netherlands (CGN), the following agreement shall be signed in advance by any recipient of material conserved by CGN that is collected in Jordan and exchanged with CGN as part of the collaboration between NCARE and CGN.

ARTICLE 1 — PARTIES TO THE AGREEMENT

1.1 This Agreement is:
BETWEEN: National Center for Agricultural Research & Extension (NCARE), Dr F. AL-Sheyab, fawzish@ncare.gov.jo (hereinafter referred to as "the Provider")
AND: Centre for Genetic Resources, the Netherlands (CGN), Wageningen, the Netherlands, Ir S-J. Hiemstra, sipkejoost.hiemstra@wur.nl (hereinafter referred to as "the Recipient").
1.2 The parties to this Agreement hereby agree as follows:

ARTICLE 2 — DEFINITIONS

In this Agreement the expressions set out below shall have the following meaning:
"*Available without restriction*": a Product is considered to be available without restriction to others for further research and breeding when it is available for research and breeding without any legal or contractual obligations, or technological restrictions, that would preclude using it in the manner specified in Article 5.1 of this Agreement.
"*Genetic material*" means any material of plant origin, including reproductive and vegetative propagating material, containing functional units of heredity.
"*Plant Genetic Resources for Food and Agriculture*" means any genetic material of plant origin of actual or potential value for food and agriculture.
"*Plant Genetic Resources for Food and Agriculture under Development*" means material derived from the Material, and hence distinct from it, that is not yet ready for commercialization and which the developer intends to further develop or to transfer to another person or entity for further development. The period of development for the Plant Genetic Resources for Food and Agriculture

underDevelopment shall be deemed to have ceased when those resources are commercialized as a Product.
"*Product*" means Plant Genetic Resources for Food and Agriculture that incorporate the Material or any of its genetic parts or components that are ready for commercialization, excluding commodities and other products used for food, feed and processing.
"*Sales*" means the gross income resulting from the commercialization of a Product or Products, by the Recipient, its affiliates, contractors, licensees and lessees.
"*To commercialize*" means to sell a Product or Products for monetary consideration on the open market, and "commercialization" has a corresponding meaning. Commercialization shall not include any form of transfer of Plant Genetic Resources for Food and Agriculture under Development.

ARTICLE 3 — SUBJECT MATTER OF THE MATERIAL TRANSFER AGREEMENT

The Plant Genetic Resources for Food and Agriculture originating from Jordan and specified in *Annex 1* to this Agreement (hereinafter referred to as the "Material") and the available related information referred to in Article 4b and in *Annex 1* are hereby transferred from the Provider to the Recipient subject to the terms and conditions set out in this Agreement.

ARTICLE 4 — RIGHTS AND OBLIGATIONS OF THE PROVIDER

The Provider undertakes that the Material is transferred in accordance with the following provisions:
a) Access shall be accorded expeditiously, without the need to track individual accessions and free of charge, or, when a fee is charged, it shall not exceed the minimal cost involved;
b) All available passport data and, subject to applicable law, any other associated available non-confidential descriptive information, shall be made available with the Plant Genetic Resources for Food and Agriculture provided;
c) Access to Plant Genetic Resources for Food and Agriculture under Development, including material being developed by farmers, shall be at the discretion of the developer, during the period of its development;
d) Access to Plant Genetic Resources for Food and Agriculture protected by intellectual and other property rights shall be consistent with relevant international agreements, and with relevant national laws.

The Provider shall periodically inform the Government of Jordan about the Material Transfer Agreements entered into, according to an agreed schedule.

ARTICLE 5 — RIGHTS AND OBLIGATIONS OF THE RECIPIENT

5.1 The Recipient undertakes that the Material shall be used or conserved only for the purposes of research, breeding and training for food and agriculture. Such purposes shall not include chemical, pharmaceutical and/or other non-food/feed industrial uses.

5.2 The Recipient shall not claim any intellectual property or other rights that limit the facilitated access to the Material provided under this Agreement, or its genetic parts or components, in the form received from the Provider.

5.3 In the case that the Recipient transfers the Material supplied under this Agreement toanother person or entity (hereinafter referred to as "the subsequent recipient"), the Recipient shall

a) do so under the terms and conditions of this Material Transfer Agreement, through a new material transfer agreement; and

b) notify theGovernment of Jordan, through the Centre for Genetic Resources, the Netherlands, in accordance with Article 4c.

On compliance with the above, the Recipient shall have no further obligations regarding the actions of the subsequent recipient.

5.4 In the case that the Recipient transfers a Plant Genetic Resource for Food and Agricultureunder Development to another person or entity, the Recipient shall:

a) do so under the terms and conditions of this Material Transfer Agreement, through a new material transfer agreement, provided that Article 4a of this Material Transfer Agreement shall not apply;

b) identify, in *Annex 1* to the new material transfer agreement, the Material received from the Centre for Genetic Resources, the Netherlands, and specify that the Plant Genetic Resources for Food and Agriculture under Development being transferred are derived from the Material;

c) notify the Government of Jordan, through the Centre for Genetic Resources, the Netherlands, in accordance with Article 4c; and

d) have no further obligations regarding the actions of any subsequent recipient.

5.5 Entering into a material transfer agreement under paragraph 5.4 shall be without prejudice tothe right of the parties to attach additional conditions, relating to further product development, including, as appropriate, the payment of monetary consideration.

5.6 In the case that the Recipient commercializes a Product that is a Plant Genetic Resourcefor Food and Agriculture and that incorporates Material as referred to in

Article 3 of this Agreement, and where such Product is not available without restriction to others for further research and breeding, the Recipient shall pay a fixed percentage of the Sales of the commercialized Product to the Government of Jordan, in accordance with *Annex 2* to this Agreement.

5.7 In the case that the Recipient commercializes a Product that is a Plant Genetic Resourcefor Food and Agriculture and that incorporates Material as referred to in Article 3 of this Agreement and where that Product is available without restriction to others for further research and breeding, the Recipientis encouraged to make voluntary payments to the Government of Jordan, in accordance with *Annex 2* to this Agreement.

5.8 The Recipient shall make available to theGovernment of Jordan, through the Centre for Genetic Resources, the Netherlands, all non-confidential information that results from research and development carried out on the Material, and is encouraged to share non-monetary benefits that result from such research and development. After the expiry or abandonment of the protection period of an intellectual property right on a Product that incorporates the Material, the Recipient is encouraged to place a sample of this Product into a collection that is identified by the Government of Jordan, for research and breeding.

5.9 A Recipient who obtains intellectual property rights on any Products developed from theMaterial or its components, obtainedthrough this Material Transfer Agreement, and assigns such intellectual property rights to a third party, shall transfer the benefit-sharing obligations of this Agreement to that third party.

ARTICLE 6 — APPLICABLE LAW

The applicable law shall be General Principles of Law, including the UNIDROIT Principles of International Commercial Contracts 2004.

ARTICLE 7 — DISPUTE SETTLEMENT

7.1 Dispute settlement may be initiated by the Provider or the Recipient .

7.2 Any dispute arising from this Agreement shall be resolved in the following manner:

a) Amicable dispute settlement: The parties shall attempt in good faith to resolve the dispute by negotiation.

b) Mediation: If the dispute is not resolved by negotiation, the parties may choose mediation through a neutral third party mediator, to be mutually agreed.

c) Arbitration: If the dispute has not been settled by negotiation or mediation, any party maysubmit the dispute for arbitration under the Arbitration Rules of

an international body as agreed by the parties to the dispute. Failing such agreement, the dispute shall be finally settled under the Rules of Arbitration of the International Chamber of Commerce, by one or more arbitrators appointed in accordance with the said Rules. Either party to the dispute may, if it so chooses, appoint its arbitrator from such list of experts as the Governing Body may establish for this purpose; both parties, or the arbitrators appointed by them, may agree to appoint a sole arbitrator, or presiding arbitrator as the case may be, from such list of experts. The result of such arbitration shall be binding.

ARTICLE 8 — ADDITIONAL ITEMS

Warranty

8.1 The Provider makes no warranties as to the safety of or title to the Material, nor as to theaccuracy or correctness of any passport or other data provided with the Material. Neither does it make any warranties as to the quality, viability, or purity (genetic or mechanical) of the Material being furnished. The phytosanitary condition of the Material is warranted only as described in any attached phytosanitary certificate. The Recipient assumes full responsibility for complying with the recipient nation's quarantine and biosafety regulations and rules as to import or release of genetic material.

ARTICLE 9 — SIGNATURE/ACCEPTANCE

The Provider and the Recipient herewith sign this Agreement.

I, (F. AL-Sheyab), represent and warrant that I have the authority to execute this Agreement on behalf of the Provider and acknowledge my institution's responsibility and obligation to abide by the provisions of this Agreement, both by letter and in principle, in order to promote the conservation and sustainable use of Plant Genetic Resources for Food and Agriculture.

Signature..... Date 8.02.2017

I, (S-J. Hiemstra), represent and warrant that I have the authority to execute this Agreement on behalf of the Recipient and acknowledge my institution's responsibility and obligation to abide by the provisions of this Agreement, both by letter and in principle, in order to promote the conservation and sustainable use of Plant Genetic Resources for Food and Agriculture.

Signature..... Date 8-02-2017

WAGENINGEN UR
WOT-UNIT CGN

D. Khaled Aboulaila, National herbarium, NCARG
Director of Plant Biodiversity Directorate Nafsha

Annex 1

LIST OF MATERIALS PROVIDED

This *Annex* contains a list of the Material provided under this Agreement, including the associated information referred to in Article 4b.
This information is either provided below or can be obtained at the following website: www.cgn.wur.nl .
The following information is included for each Material listed: all available passport data and, subject to applicable law, any other associated, available, non-confidential descriptive information.
(*List)*

See appendix 4 of the present report

Annex 2

RATE AND MODALITIES OF PAYMENT UNDER ARTICLE 5.6 OF THIS AGREEMENT

1. If a Recipient, its affiliates, contractors, licensees, and lessees, commercializes a Product or Products, then the Recipient shall pay one point-one percent (1.1 %) of the Sales of the Product or Products less thirty percent (30%); except that nopayment shall be due on any Product or Products that:

(a)are available without restriction to others for further research and breeding in accordance with Article 2 of this Agreement;

(b)have been purchased or otherwise obtained from another person or entity who either has already made payment on the Product or Products or is exempt from the obligation to make payment pursuant to subparagraph (a) above;

(c) are sold or traded as a commodity.

2. Where a Product contains a Plant Genetic Resource for Food and Agriculture accessedfrom the Material originating from the territory of Jordanunder two or more material transfer agreements only one payment shall be required under paragraph 1 above.

3. The Recipient shall submit to the Government of Jordan, within sixty (60) days after each calendaryear ending December 31st, an annual report setting forth:

(a)the Sales of the Product or Products by the Recipient, its affiliates, contractors, licensees and lessees, for the twelve (12) month period ending on December 31st;

(b)the amount of the payment due; and

(c) information that allows for the identification of any restrictions that have given rise to the benefit-sharing payment.

4. Payment shall be due and payable upon submission of each annual report. All payments due tothe Government of Jordanshall be payable in *United States dollars (US$)*of the following account established by the Government of Jordan:

Account holder: The National Center for Agricultural Research and Extension (NCARE)

Bank Name: ARAB BANK PLC

Bank address: AMMAN-BAQA BRANCH, PO BOX 144186, ZIP CODE: 11814 Amman

Bank contact details: tel: 00962-6-4725743, 00962-6-4725741, 00962-6-5371147; fax: 00962-6-4725742

Swift code: ARABJOAX100

Account Number: 142/181960-7/510

IBAN JO55 ARAB 1420 0000 0014 2181 9605 10

Ministry Of Agriculture
National Center for Agricultural
Research and Extension

وزارة الزراعة
المركز الوطني للبحث و الارشاد
الزراعي

NO. 5/16/B/997
Date 8/4/2015

الرقم : .....
التاريخ : ...../٤/٢٠١٥.....
الموافق : .....

Declaration of Prior Informed Consent

The undersigned, acting on behalf of/in his capacity of the genetic resources for food and agriculture occurring in Jordan designated competent national authority, here with declares to provide Prior Informed Consent to the Centre for Genetic Resources, the Netherlands of Wageningen University and Research Centre, represented by its director Dr. Bert Visser, for the purpose of obtaining, maintaining and distributing plant genetic resources, collected from Jordan's territory in the framework of the agreed joint capacity building project starting 1 January 2015 and expected to end not later than December 2020, according to the conditions set out in the duly signed Memorandum of Understanding concerning conservation of plant genetic resources for food and agriculture occurring in Jordan (appendix).

Director General

Dr. Fawzi Alsheyab

Hashemite Kingdom Of Jordan
National Center For Agricultural
Research And Extension

هاتف : ٤٧٢٥٠٧١ ٦ (٩٦٢+) – فاكس: ٤٧٢٦٠٩٩ ٦ (٩٦٢+)– ص . ب: (٦٣٩)- البقعة ١٩٣٨١ الأردن
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MINISTRY OF ENVIRONMENT

No. .....
Date: 14/6/2017

To whom it may concern

Hereby I, Eng. Raed Bani Hani, in my capacity of the Jordan ABS National Focal Point, approve the Material Transfer Agreement (date: 8/2/2017; see attachment) which has been slightly modified to clarify some points and has been signed by NCARE and CGN.

Yours truthfully

Eng. Raed Bani Hani
Nature Protection Director
Ministry of Environment
PO Box 1408
Amman 11941
Jordan

THE HASHEMITE KINGDOM OF JORDAN-AMMAN

Appendix 2 Expedition collecting form

Jordan - the Netherlands *Lactuca* expedition 2017

Team/collector(s) ……*Collecting number*……..

Date……*Photo number*……

Crop name……*Cultivar name*……..

Latin species name……

Locality……

……

Latitude……*Longitude*……*Altitude*……

Number of plants sampled:

Topography…swamp…flood plain…level…undulating…hilly…steep…mountainous

Biological status of accession

100) Wild

200) Weedy

300) Traditional cultivar/landrace

500) Advanced/improved cultivar

Collecting/acquisition source

10) Wild habitat
11) Forest/woodland
12) Shrubland
13) Grassland
14) Desert/tundra
15) Aquatic habitat

20) Farm or cultivated habitat
21) Field
22) Orchard
23) Backyard, kitchen or home garden
24) Fallow land
25) Pasture

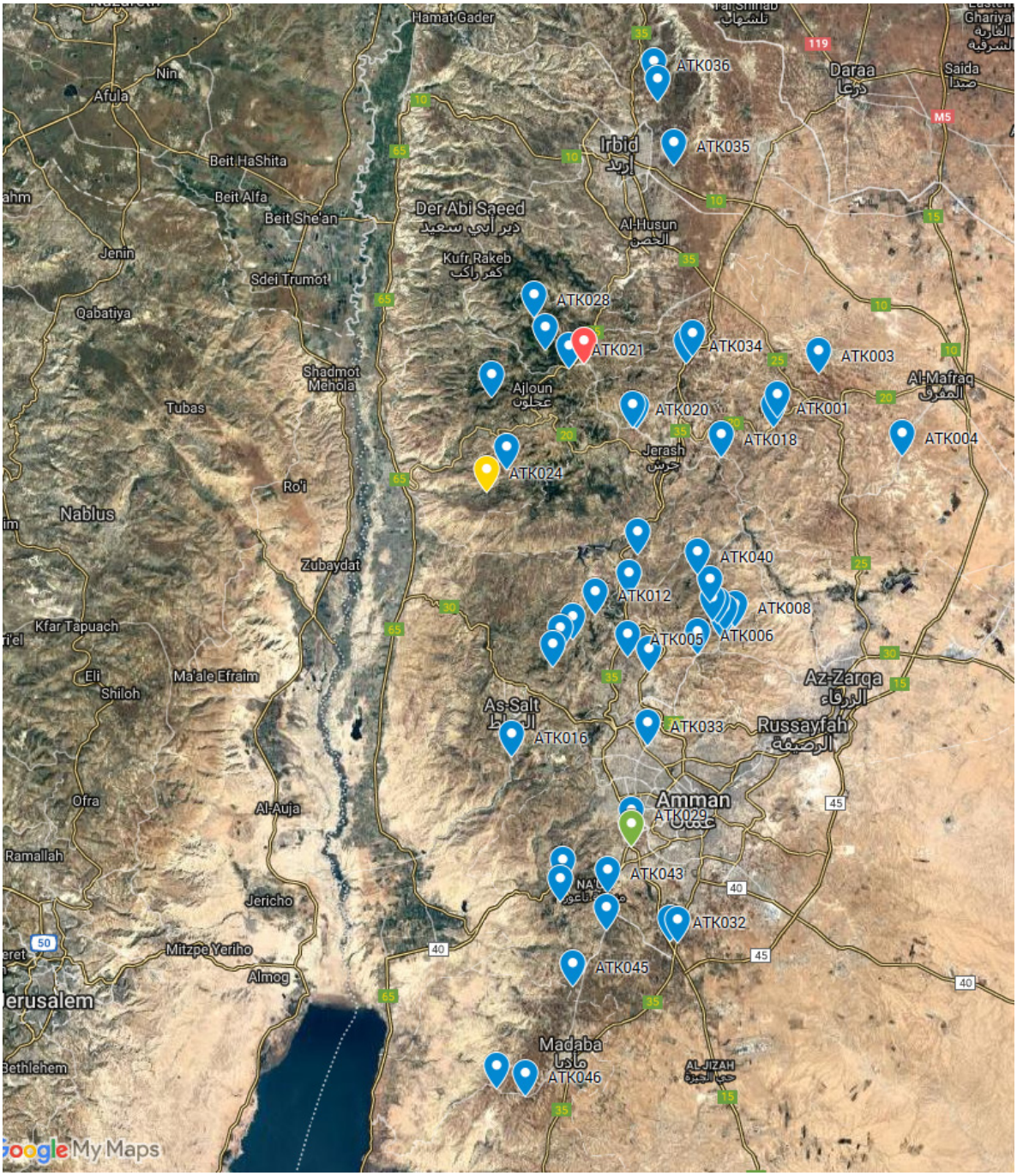
30) Market or shop

60) Weedy, disturbed or ruderal habitat
61) Roadside
62) Field margin

REMARKS
(diseases, pests, other)

Appendix 3a Map of locations where collecting took place in North-Middle Jordan

Collecting numbers (ATKxx) are indicated for the *Lactuca* material collected; blue marker: *Lactuca aculeata*, red: *Lactuca serriola/saligna*, yellow: *Lactuca serriola*, green: *Lactuca saligna.*



Appendix 3b Map of locations where collecting took place in Middle-South Jordan

Collecting numbers (ATKxx) are indicated for the *Lactuca* material collected; blue marker: *Lactuca aculeat*a, brown: *Lactuca undulata*, purple: *Lactuca orientalis.*

Appendix 4 Passport data of *Lactuca* accessions (ATK xxx) collected in Jordan

RNR: receipt number CGN, date: day-month-year, latitude and longitude determined via GPS: mapdatum WGS84, position format for longitude and latitude: hddd.ddddd', altitude in meters; population type: W: wild material.

RNR	collecting number	country	photo map	photo nr	date	Lactuca species name given during expedition	nearest locality name	latitude	longitude	altitude	population type	topography	collecting source	number of plants sampled	number of double glassine bags	number of single glassine	population size	remarks
170005	ATK001	JOR	108	732-734	12-9-2017	aculeata	road Jerash-Mafraq	32.30279	36.01570	757	W	hilly	roadside	26	23	3	> 150	
170006	ATK002	JOR	108	735	12-9-2017	aculeata	road Jerash-Mafraq	32.31113	36.02037	759	W	undulating	orchard	10	4	6	50	olive orchard
170007	ATK003	JOR	108	736,737	12-9-2017	aculeata	Al Manshiyah	32.35178	36.06551	864	W	undulating	orchard	30	29	1	>250	olive orchard
170008	ATK004	JOR	108	738,739	12-9-2017	aculeata	Bala'ama	32.27523	36.15711	752	W	level	roadside	1	1	0	1	
170009	ATK005	JOR	108	740	13-9-2017	aculeata	Baqa'a	32.08833	35.85496	632	W	level	roadside	4	4	0	<5	
170010	ATK006	JOR	108	741,743	13-9-2017	aculeata	Um Rumanah	32.09116	35.93239	792	W	level	field	20	20	0	50	barley
170011	ATK007	JOR	108	744,745	13-9-2017	aculeata	Birayn-1	32.10503	35.95922	699	W	undulating	orchard	16	15	1	>200	
170012	ATK008	JOR	108	746	13-9-2017	aculeata	Birayn-2	32.11635	35.97340	633	W	level	roadside	6	3	3	30	small plants along road
170013	ATK009	JOR	108	747	13-9-2017	aculeata	Birayn-3	32.11323	35.96278	670	W	level	roadside	4	0	4	<10	
170014	ATK010	JOR	108	749	13-9-2017	aculeata	A'alouk-1	32.11973	35.95362	701	W	level	roadside	34	25	9	500	
170015	ATK011	JOR	108	750,751	13-9-2017	aculeata	A'alouk-2	32.12255	35.94846	774	W	undulating	backyard	67	67	0	500	
170016	ATK012	JOR	108	754,755	14-9-2017	aculeata	Al Selehee	32.12798	35.82002	591	W	hilly	fallow land	28	22	6	100	
170017	ATK013	JOR	108	757	14-9-2017	aculeata	Rmaymeen	32.10432	35.79526	627	W	level	field	7	3	4	<25	
170018	ATK014	JOR	108	758	14-9-2017	aculeata	Al Waseyah	32.09373	35.78148	778	W	level	roadside	1	1	0	1	
170019	ATK015	JOR	108	760	14-9-2017	aculeata	Mudhary	32.07962	35.77234	944	W	undulating	field margin	48	26	22	>250	
170020	ATK016	JOR	108	761,762	14-9-2017	aculeata	As Salt	31.99564	35.72765	624	W	hilly	roadside	48	40	8	>1000	
170021	ATK017	JOR	108	763,764	17-9-2017	aculeata	Mastaba	32.14489	35.85623	563	W	level	roadside	29	1	28	70	
170022	ATK018	JOR	108	765	17-9-2017	aculeata	Al Ganeyeh	32.27396	35.95801	761	W	undulating	roadside	62	18	44	250	mostly small plants
170023	ATK019	JOR	108	766,767	17-9-2017	aculeata	Souf-1	32.30115	35.86426	903	W	hilly	orchard	7	3	4	20	olive orchard
170024	ATK020	JOR	108	770	17-9-2017	aculeata	Souf-2	32.30183	35.86074	918	W		orchard	10	3	7	10	olive orchard
170025	ATK021	JOR	108	774,775	17-9-2017	aculeata	Ebbein-1	32.35685	35.79003	1126	W	level	field	24	5	19	100	playing field; previous population destroyed due to building houses
170026	ATK022	JOR	108	776-780	17-9-2017	serriola/saligna	Ebbein-2	32.36134	35.80686	1087	W	level	roadside	8	2	6	>100	serr/sal mix population?
170027	ATK023	JOR	108	781	18-9-2017	aculeata	Anjara	32.36136	35.80688	1214	W	hilly	backyard	3	0	3	10	
170028	ATK024	JOR	108	782,783	18-9-2017	serriola	Wadi Rajib	32.24238	35.70015	333	W	undulating	orchard	21	14	7	>1000	
170029	ATK025	JOR	108	784,785	18-9-2017	aculeata	Rajib	32.26311	35.72161	780	W	undulating	field margin	58	37	21	500	
170030	ATK026	JOR	108	790,791	18-9-2017	aculeata	Ajlun castle	32.32988	35.70545	694	W	level	orchard	41	24	17	200	grapes
170031	ATK027	JOR	108	793	18-9-2017	aculeata	Birgish	32.37384	35.76401	1164	W	hilly	roadside	16	6	10	50	
170032	ATK028	JOR	108	794	18-9-2017	aculeata	Oyoun -Rasun	32.40405	35.75244	746	W	level	roadside	2	2	0	10	
170033	ATK029	JOR	108	796	19-9-2017	aculeata	Amman-Airport road-1	31.92390	35.85966	909	W	concrete slope	roadside	14	3	11	20	
170034	ATK030	JOR	108	797,799	19-9-2017	aculeata	Amman-Airport road-2	31.91127	35.85948	910	W	undulating	roadside	49	44	5	>1000	
170035	ATK031	JOR	108	800-802	19-9-2017	saligna	Amman-Airport road-2a	31.91127	35.85948	910	W	undulating	roadside	26	2	24	100	
170036	ATK032	JOR	108	805,807,809	19-9-2017	aculeata	Amman-Airport road-3	31.82317	35.90171	801	W	level	roadside	56	29	27	>1000	opposite IKEA
170037	ATK033	JOR	108	810	19-9-2017	aculeata	Amman	32.00602	35.87732	1001	W	level	roadside	11	7	4	40	parking University of Jordan; recently destroyed large population
170038	ATK034	JOR	108	820,822,823	20-9-2017	aculeata	Qafqafa	32.36124	35.91999	834	W	level	roadside	28	18	10	100	along excavation, also with flowering plants
170039	ATK035	JOR	108	825-827	20-9-2017	aculeata	Huwara	32.54588	35.90644	548	W	level	field	44	26	18	300	barley field
170040	ATK036	JOR	108	829	20-9-2017	aculeata	Kharja-Harima	32.62116	35.88363	524	W	level	field margin	22	8	14	50	
170041	ATK037	JOR	108	830,831	20-9-2017	aculeata	Harima	32.60441	35.88777	513	W	level	roadside/orchard	17	5	12	50	olive orchard
170042	ATK038	JOR	108	832	20-9-2017	aculeata	Balila	32.36854	35.9272	805	W	level	roadside	4	2	2	10	
170043	ATK039	JOR	108	833	21-9-2017	aculeata	A'alouk-3	32.13965	35.94584	667	W	undulating	roadside	3	1	2	5	
170044	ATK040	JOR	108	834	21-9-2017	aculeata	Mastaba	32.16502	35.93213	594	W		roadside	2	2	0	<5	
170045	ATK041	JOR	108	835	21-9-2017	aculeata	Jubba	32.18431	35.86641	595	W	level	roadside	45	7	38	70	
170046	ATK042	JOR	108	837	21-9-2017	aculeata	Abu Hamid	32.07390	35.87806	856	W	level	roadside	56	37	19	200	
170047	ATK043	JOR	108	838,839	24-9-2017	aculeata	Na'our-1	31.86836	35.83385	935	W	level	roadside	5	1	4	20	
170048	ATK044	JOR	108	840	24-9-2017	aculeata	Na'our-2	31.83374	35.83257	887	W	level	field	7	3	4	50	
170049	ATK045	JOR	108	841,842	24-9-2017	aculeata	Mushagar	31.67814	35.74294	816	W	level	roadside	59	15	44	>500	
170050	ATK046	JOR	108	843	24-9-2017	aculeata	Madaba	31.78127	35.79528	799	W	level	roadside	1	1	0	1	road to Ma'ein springs
170051	ATK047	JOR	108	864,865	24-9-2017	aculeata	Hisban road	31.87753	35.78407	623	W	level	roadside	2	0	2	10	
170052	ATK048	JOR	108	866	24-9-2017	aculeata	Hisban-Marj Al Haman	31.85981	35.78091	827	W	level	roadside	1	1	0	1	
170053	ATK049	JOR	108	867,872	25-9-2017	aculeata	Yadodeh	31.82115	35.91013	801	W	level	roadside	25	1	24	50	
170054	ATK050	JOR	108	881-884	25-9-2017	aculeata	Karak-1	31.18731	35.78879	923	W	level	roadside	51	8	43	>500	entrance Karak from desert highway
170055	ATK051	JOR	108	889-892	25-9-2017	aculeata	Karak-2	31.16735	35.73633	993	W	level	roadside	6	2	4	20	towards city centre
170056	ATK052	JOR	108	893,894	25-9-2017	aculeata	Moacta	31.08795	35.68903	1156	W	level	roadside	60	35	25	100	
170057	ATK053	JOR	108	904,905	25-9-2017	aculeata	Tafila	30.85448	35.65492	1195	W	undulating	backyard	52	17	35	300	Christmas aculeata'
170058	ATK054	JOR	108	913	26-9-2017	aculeata	Shobak-Najil	30.50528	35.52942	1438	W	level	field margin	62	33	29	300	
170059	ATK055	JOR	108	917-920	26-9-2017	orientalis	Naqab	30.08521	35.43207	1649	W	undulating	desert	30			>1000	bulk sampled; very small spiny plants, also flowering
	ATK-veg	JOR	108	844-847	24-9-2017	aculeata	Madaba-south	31.68537	35.7113									vegetative growth of meristems in inflorescence?
	ATK-und	JOR	108	909,911	26-9-2017	undulata	Shobak-castle	30.53260	35.61245									completely withered

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Wageningen University & Research
CGN report 40

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