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Identification of new dual-purposed barley genotypes for the MENA region

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Introduction

Integrated crop and livestock farming is the predominant system in the Drylands of the MENA (Middle East and North Africa) region. Used traditionally by the most vulnerable farmers, these agroecosystems are particularly threatened by Climate Change. In these agricultural systems, farmers depend on forages and straw to feed their animals during summer and winter seasons (Ryan et al., 2008) and with the recent drought events and the over-use of pastoral lands fodder and forage prices increasing (FAOSTAT 2020). The ICARDA Global barley breeding program is aiming to develop new breeding lines that produce more forage with limited yield and straw penalty and high feed quality. Dual purpose strategies adapted to the environment can be up to 12% more profitable than grain alone (Ates et al., 2018) under optimum rainfall and can contribute to the whole system stability under drought by providing nutritious feed and forage sources for small ruminants when other sources are not available. In the present study we aim to test a set of 37 elite ICARDA barley genotypes and 3 commercial checks in the field in Morocco under simulated grazing and conventional conditions to determine their forage production, yield and biomass regeneration capacity and straw quality.

Experimental set-up

A set of 37 elite ICARDA barley genotypes and 3 commercial checks was assembled and tested in the field in Morocco under simulated grazing and conventional conditions in the 2019/20 cropping season. Of the 37 lines, 9 (Lines 4-12; Table 1) were selected based on forage production and regeneration capacity from a similar experiment carried out in the 2018/19 season (Verma 2019). The remaining 28 (Lines 13-40; Table 1) were new elite lines selected based on yield and biomass production in 4 locations in Morocco, Lebanon and India in the 2018/19 season.

The set was evaluated in a field trial consisting in a replicated Alpha-Lattice design with 80 plots 5 m-long, 6 rows and 20cm between rows. Fifty days after emergence, each plot was divided in 2 sub-plots of 2.5 m-long and a simulated grazing treatment was applied to one sub-plot per plot. The treatment consisted in cutting the aboveground biomass (leaves and shoots) ca. 5cm above the ground level (Figure 1). The fresh samples are then weighed and a subsample of each is dried in an air-oven at 70C for 2 days to determine the moisture content and the dry weight of the green cuts. Full irrigation was planned although only limited irrigation could be applied due to the severe drought in Morocco with 230mm during the crop cycle and only 28mm from mid-December to mid-March.

Heading date and maturity date were collected from all plots, cut and control, as the date when 50% of the spikes were out the shoot (Zadoks 55) and the date when 50% of the peduncles turned yellow (Zadoks 87). Finally, the plots were harvested at ripening and grain and biomass yield were determined. The set was also scored for diseases present under natural infection, particularly Net Form of Net Blotch and Spot Form of Net Blotch.

Straw samples from all the plots were collected and transferred to the ICARDA-Rabat quality laboratory to determine feed quality. The samples were grinded and analyzed with near-Infrared spectroscopy in a FOSS DS2500 NIRS (FOSS, Denmark) calibrated for feed quality traits in collaboration with ILRI feed quality laboratory (ILRI; Kenya). Thus, straw organic matter (OM, %), ash, crude protein (CP, %), dry matter (DM, %), acid detergent fiber (ADF, %), acid detergent lignin (ADL, %), neutral detergent fiber (NDF, %). Hemicellulose was calculated as (NDF-ADF), and cellulose as (ADF-ADL).

All analysis were performed using Genstat v21 (VSN International 2020) and R software (R Core Team 2020) packages *statgenSTA* (van Rossum 2020a) and *statgenGxE* (van Rossum 2020b).

Forage production

The results of the cutting treatment showed significant differences between the lines in forage production ($p < 0.01$; $h^2 = 0.47$; $CV = 12.98\%$). Up to 22 testing lines produced more forage than the best check Rihane-03 (Figure 2) and the best two lines (Entries 12 and 38) produced more than 30% more forage ($p < 0.05$). Entry 12 was already identified as high forage yielding line in a previous dual-purpose treatment trial

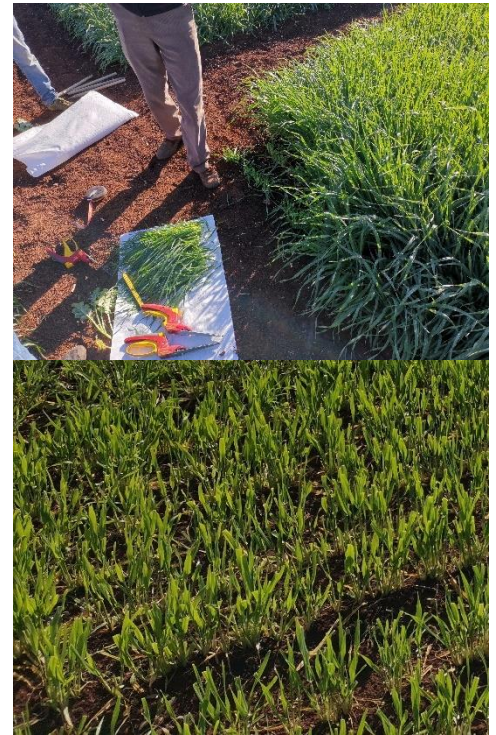


Figure 1 Green cuts treatment performed in a field trial carried out in Marchouch (Morocco) in the 2019/20 season (top picture). Regenerating barley after green cut treatment (bottom picture).

(Verma 2019) and Entry 38 is a new elite line tested in the present study for the first time under dual-purpose management.

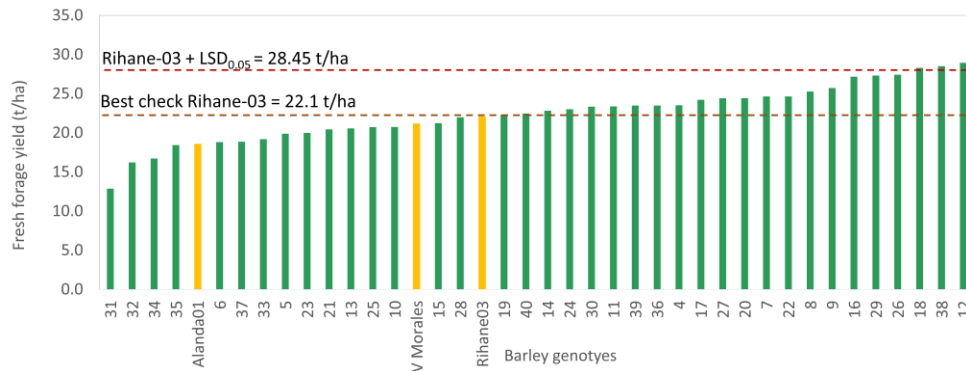


Figure 2 Forage yield of 37 ICARDA elite barley lines and 3 checks obtained from a cutting treatment in a field trial carried out in Marchouch (Morocco) in the 2019/20 cropping season. The yellow bars represent the commercial checks. The brown line represents the forage yield of the best commercial check Rihane-03 (22.1 t/ha) and the red lines represents the forage yield of Rihane-03 plus the $LSD_{0.05}$.

Phenology is probably the single most crucial moment for cereal productivity. Matching flowering time to the environmental conditions can increase resilience and performance. The results of the present study showed that forage yield and flowering time in the control plots were correlated and that early genotypes tended to produce more forage yield than later ones ($R^2=0.36$; Figure 3). However, large diversity could be found within the set. In fact, the highest forage yielding genotype (Entry 12) flowered later than 2 of the checks and than the average of the trial. This result suggest that, although early flowering can be a desired trait for forage production the two traits are not necessarily linked and therefore plant breeders can manipulate both independently to develop lines adapted to the environment and with high forage production.

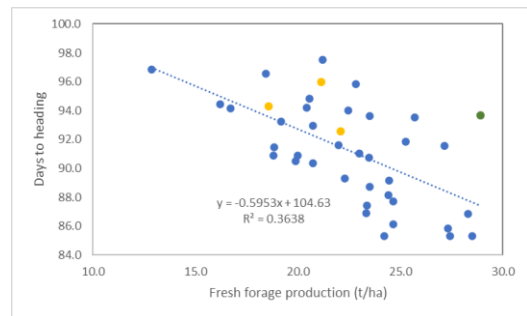


Figure 3 Biplot comparing the days to heading and the fresh forage production of 37 ICARDA elite barley lines and 3 checks obtained from a cutting treatment in a field trial carried out in Marchouch (Morocco) in the 2019/20 cropping season. Yellow dots represent the commercial checks and the green dot represents the highest forage yielding genotype.

Disease resistance

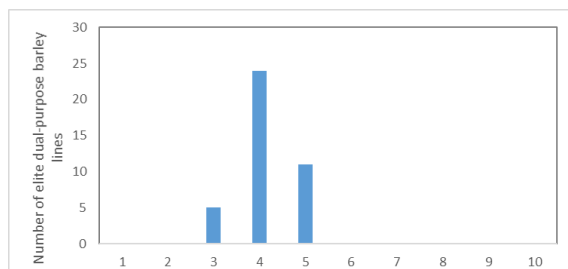


Figure 4 Reaction to Spot Form of Net Blotch (numerical scale) of 37 ICARDA elite barley lines and 3 checks tested in a field trial carried out in Marchouch (Morocco) under conventional management in the 2019/20 cropping season.

Biotic stress can hinder both forage and grain production. For this reason, the lines selected to be tested under dual-purpose management need to have a minimum level of resistance to common diseases. Among the different disease hindering barley production in Morocco, net blotch, both in its net (NFNB; *Pyrenophora teres f. teres*) and spot forms (SFNB; *Pyrenophora teres f. maculata*), is one of the most important. Yield losses up to 44% have reported in the country due to spot-form of net blotch coupled with grain quality reductions (McLean et al., 2009). In Marchouch and under natural infection all

the lines tested proved to be Moderately Resistant to Spot Form of Net Blotch, the prevalent barley disease in the area.

Feed and fodder production and regeneration

In general terms, the dual-purpose management resulted in a yield and biomass penalty of 19.1% (0.7 t/ha) and 37.6% (3.7 t/ha) on average in the dual-purpose management as compare to the control respectively (Figure 5). The average loss in dry biomass at the end of the cycle due to the dual-purpose management (3.7 t/ha) was similar to the average forage weight produced adjusted to 12% moisture content (3.3 t/ha).

A significant, although minor, negative association between green forage production and biomass and grain yield both under control (data not shown) and dual-purpose management was found ($R^2 < 0.3$ for both, Figure 6). The regeneration capacity of the lines evaluated - calculated as the percentage of biomass and grain yield loss per genotype between the two managements - ranged from 27 to 48% for biomass and from 0 to 34% for grain yield (Table 1). No significant relationship was found between forage production and the biomass regeneration capacity of the genotypes tested (data not shown) while a minor negative association ($R^2 < 0.1$; $p = 0.478$) was found with grain yield regeneration.

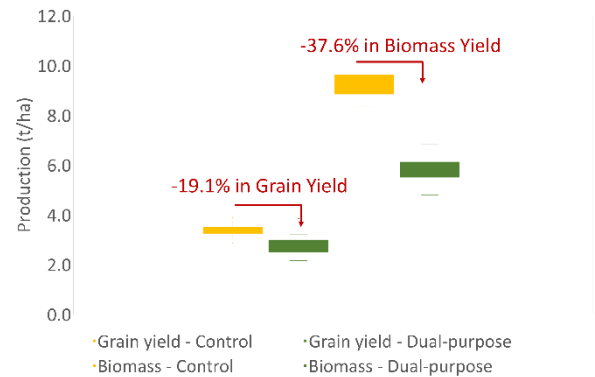


Figure 5 Box plot of the grain yield and biomass production of 37 ICARDA elite barley lines and 3 checks tested in a field trial carried out in Marchouch (Morocco) under conventional and dual-purpose managements in the 2019/20 cropping season.

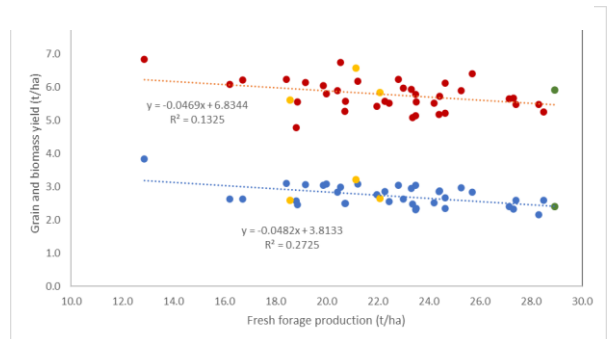


Figure 6 Biplot comparing the the fresh forage production and the biomass (red dots) and grain (blue dots) production of 37 ICARDA elite barley lines and 3 checks obtained from a cutting treatment in a field trial carried out in Marchouch (Morocco) in the 2019/20 cropping season.

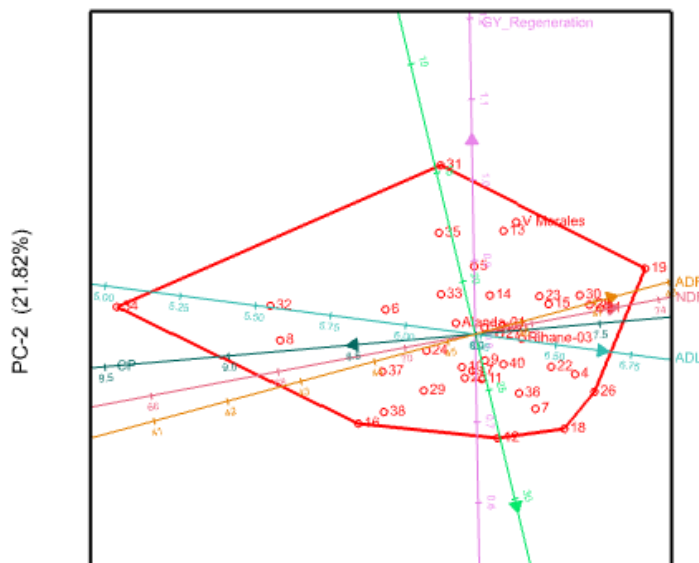


Figure 7 Biplot of the first two principal components of an analysis carried out with NDF, ADL, ADF, CP, forage production and yield regeneration.

Feed quality

The feed quality of the straw harvested at the end of the cycle from each plot of the dual-purpose management was analyzed. All the traits analyzed were significant and the heritabilities ranged from 0.51 for the ash content to 0.73 for NDF. The results showed that feed quality was independent from forage production or other variables

analyzed (Figure 7). The commercial checks showed generally low feed quality, particularly, low crude protein and high ADL, ADF and NDF as compared with the average of the trial. On the other hand the 3 genotypes showed high feed quality, entries 34, 32 and 8. While entries 32 and 32 showed low forage production as compared to the commercial checks, entry 8 was among the highest forages producers (Figure 2).

Conclusions

Dual-purpose management in barley is an agronomic strategy that has been used in the MENA region for thousands of years. This strategy has proven to increase the profits per hectare when applied in environments with more than 300mm of rainfall (Ates et al., 2018). In the present study a series of elite barley lines were tested under this management to evaluate their forage production, regeneration capacity and feed quality. The results showed that up to 30% more forage production can be achieved with the new elite barley genotypes as compared with the commercial checks. However, forage production showed a negative association with the yield and biomass production of the lines. This result suggests that increasing forage production could result in a decrease in yield and biomass. However, this result can be partially explained by the environmental conditions of the season. Morocco suffered one of the worst drought and heat events in the last decades (only 28mm from mid-December to mid-March and with unusually high temperatures) while from March to June, more than 180mm of rainfall were registered. Lines producing high forage yield early in the cycle would have their water needs increased to maintain the biomass and under this conditions, would suffer a higher stress while the late flowering lines producing lower forage would be more tolerant. In order to clarify the true potential of the lines under a dual purpose management, a new trial would be needed under secured water input and with different cutting times to confirm the conclusions. Also, the high forage production lines could be useful in other agronomic strategies besides dual-purpose. For instance, an increase in the interest for hydroponic forage production has been seen in the MENA region in the last years. These high early vigor lines could fit these schemes and provide forage to farmers with this technology. In fact, a set of the high forage producing lines from this study will be tested in Saudi Arabia and Morocco for this aim.

The cutting treatment resulted in considerable average yield and biomass penalties as compared with the control treatment (conventional management). A similar effect was observed in a study carried out in Turkey under stressed conditions (Ates et al., 2018). However, the biomass penalty was similar to the forage production, suggesting that the loss in final biomass could be interpreted as a reallocation of the biomass production within the season. This result suggests that, even in sub-optimal years, farmers living in regions where forage is scarce in winter could use the high forage yielding varieties as a way to optimize the feed distribution to their livestock within the year. Yield penalties were instead highly variable, with some lines showing no or reduced penalty. For instance Entry 8 (Rhn//Bc/Coho/3/DeirAlla106//Api/EB89-8-2-15-4/5/CM67/3/Apro//Sv02109/Mari/4/Carbo/6/IPA7), a high forage yielding line, showed a yield penalty of only 8%. This line showed also high feed quality as compared to the commercial checks and has been shared with NARS for validation.

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Supplementary Table 1: Elite barley lines tested under conventional and dual-purpose management in a field trial in Marchouch (Morocco) in the 2019/20 cropping season.

| Entry | Cross name | Sel. History |
|-------|--|--|
| 1 | Alanda01 | Check 1 |
| 2 | Rihane03 | Check 2 |
| 3 | V Morales | Check 3 |
| 4 | Rhn//Bc/Coho/3/DeirAlla106//Api/EB89-8-2-15-4/5/CM67/3/Apro//Sv02109/Mari/4/Carbo/6/Hma-02//11012-2/CM67/3/Alanda/5/Rhn-03//Lignee527/NK1272/4/Lignee527/Chn-01/3/Alanda | ICB08-0291-1AP-0AP-025AP-5AUB-OKF |
| 5 | Rhn//Bc/Coho/3/DeirAlla106//Api/EB89-8-2-15-4/5/CM67/3/Apro//Sv02109/Mari/4/Carbo/6/Lignee527/NK1272//JLB70-063/3/Rhn-03 | ICB08-0292-4AP-0AP-025AP-5AUB-OKF |
| 6 | Rhn//Bc/Coho/3/DeirAlla106//Api/EB89-8-2-15-4/5/CM67/3/Apro//Sv02109/Mari/4/Carbo/6/IPA7 | ICB08-0294-6AP-0AP-025AP-2AUB-OKF |
| 7 | P.STO/3/LBIRAN/UNA80//LIGNEE640/4/BLLU/5/PETUNIA_1 | CBSS97M00850T-G-2M-1Y-2M-0Y-0AP-21MR |
| 8 | Rhn//Bc/Coho/3/DeirAlla106//Api/EB89-8-2-15-4/5/CM67/3/Apro//Sv02109/Mari/4/Carbo/6/IPA7 | ICB08-0294-17AP-0AP-025AP-5AUB-OKF |
| 9 | Lignee527/NK1272//JLB70-063/3/Alanda/Zafraa//Gloria'S'/Copal'S'/4/Gloria'S'/Copal'S'//As46/Aths/3/Rhn-03 | ICB10-0257-0AP-0AP-0MR-0MR |
| 10 | Rihane-03/3/As46/Aths*2//Aths/Lignee686/6/Rhn-03/Eldorado/5/Rhn-03//Lignee527/NK1272/4/Lignee527/Chn-01/3/Alanda | ICB08-0303-4AP-0AP-025AP-3AUB-OKF |
| 11 | CompCr229//As46/Pro/3/Srs/4/RWA-M47/5/Carbo/Hamra/4/Rhn-08/3/DeirAlla106//DL71/Strain205 | ICB08-0057-18AP-0AP-025AP-5AUB-OKF |
| 12 | Avt/Attiki//M-Att-73-337-1/3/Aths/Lignee686/4/M-Att-73-337-1/3/Mari/Aths*2//Avt/Attiki/5/Manel | ICB09-0108-0TR-0MC |
| 13 | Ishi//Rihane/221BYT7 | UCD13-032-0UCD-0UCD-0MR-0MR-10MR |
| 14 | SC 3883 K2/STANDER-BAR | ICM1213CJ34-32CJ-010CH-05CJ-5CH-0MR |
| 15 | Gloria'S'/Copal'S'//As46/Aths/3/Rhn-03/5/QB813-2/5/Aths/Lignee686/4/Rhn-03/3/Bc/Rhn//Ky63-1294 | ICB09-0510-0AP-0AP-025AUB-4AUB-0MR |
| 16 | Chn-01/CC89//Ariol/3/Lignee640/Bgs//Cel/4/Lignee527/Aths/5/Sawsan/Badia//Arar/3/Gloria'S'/Copa I'S' | ICB10-0798-0AP-025AUB-015TR-2AUB-0MR |
| 17 | Rhn-03/Asse//RWA-M54/3/Saida/4/Rihane-03/3/As46/Aths*2//Aths/Lignee686 | ICB10-0278-0AP-025AUB-015TR-2AUB-0MR |
| 18 | Lignee527/NK1272//JLB70-063/3/Bda/4/Sawsan/Badia//Arar/3/Gloria'S'/Copal'S' | ICB10-0785-0AP-025AUB-015TR-2AUB-0MR |
| 19 | UC1231//Rihane 03/UC1118 | UCD13-039-0MR-0MR-3MR |
| 20 | Doña Josefa/3-1MBN11 | RSI/ICJ11-12B1075-2CJ-05CH-05CJ-1CH-0CJ-0MR |
| 21 | UC933//Tampalpais/Rihane 03 | UCD13-030-0MR-0MR-3MR |
| 22 | Lignee527/NK1272//JLB70-063/3/Rhn-03/6/Rhn//Bc/Coho/3/DeirAlla106//Api/EB89-8-2-15-4/5/CM67/3/Apro//Sv02109/Mari/4/Carbo | ICB09-0684-0AP-0AP-025AUB-2AUB-0MR |
| 23 | ATACO/BERMEJO//HIGO/3/CALI92/ROBUST/4/PETUNIA 1/5/PETUNIA 1/CHINIA/3/ATACO/BERMEJO//HIGO/6/ZIGZIG/3/M9846//CCXX14.ARZ3/PACO/7/ESMERALDA/3/SLLO/ROBUST//QUINA/4/M104 | HIICB12-528-0TR-0TR-0MR-0MR-3MR |
| 24 | ROBUR-BAR/J.126//OWB753431D/SL3/4/GLORIA-BAR/COPAL//BEN.4D/3/S.P-B/5/1USWBSI | ICB09-1970-0AP-025AUB-2AUB-0MR |
| 25 | Rhn-03/Eldorado/5/Rhn-03//Lignee527/NK1272/4/Lignee527/Chn-01/3/Alanda/6/Maknusa | ICB09-0540-0AP-0AP-025AUB-2AUB-0MR |
| 26 | G12068 F3 13/030043 | H00011003/H03010040-0MR-0MR-3MR |
| 27 | P.STO/3/LBIRAN/UNA80//LIGNEE640/4/BLLU/5/PETUNIA 1/6/CIRU/5/LEGACY/4/TOCTE//GOB/HUMAI10/3/ATAH92/ALELI | HIICB12-393-0TR-0TR-0MR-0MR-6MR |
| 28 | UC1231//Rihane 03/UC1118 | UCD13-039-0MR-0MR-3MR |
| 29 | MSEL//LM 844/QUILMES PAMPA/3/BUCK M8.88/E.ACACIA//MSEL | RSI/ICJ11-12B0035-9CJ-05CH-05CJ-4CH-0CJ-0MR |
| 30 | MADRE SELVA/3/BREA/DL70//3*TOCTE | ICM13CH7-6CH-05CJ-010CH-0MR |
| 31 | UC1266//SuNu/Ishi | UCD13-094-0UCD-0UCD-0MR-0MR-6MR |
| 32 | UC933//Rihane/221BYT7 | UCD13-017-0UCD-0UCD-0MR-0MR-2MR |
| 33 | Gize 132/2* Ishi | UCD13-021-0UCD-0UCD-0MR-0MR-2MR |
| 34 | UC933//Rihane 03/22nd IBYT7 | UCD13-048-0MR-0MR-4MR |
| 35 | Ishi *2/Giza 132 | UCD13-071-0UCD-0UCD-0MR-0MR-2MR |
| 36 | Doña Josefa/ND25160 | RSI/ICJ11-12B1115-25CJ-05CH-05CJ-1CH-0CJ-0MR |
| 37 | ATACO/BERMEJO//HIGO/3/CALI92/ROBUST/4/PETUNIA 1/5/PETUNIA 1/CHINIA/3/ATACO/BERMEJO//HIGO/6/ZIGZIG/3/M9846//CCXX14.ARZ3/PACO/7/ESMERALDA/3/SLLO/ROBUST//QUINA/4/M104 | HIICB12-528-0TR-0MR-0MR-0MR-8MR |
| 38 | DOÑA JOSEFA/3/BREA/DL70//3*CABUYA | ICM13CH57-52CH-05CJ-010CH-0MR |
| 39 | DOÑA JOSEFA/3/BREA/DL70//3*CABUYA | ICM13CH57-104CH-05CJ-010CH-0MR |
| 40 | P.STO/3/LBIRAN/UNA80//LIGNEE640/4/BLLU/5/PETUNIA 1/6/M9846//CCXX14.ARZ3/PALTON/6/ESMERALDA/3/SLLO/ROBUST//QUINA/4/M104 | HIICB12-545-0TR-0MR-0MR-0MR-7MR |

