# Yield and Quality Performance of New Alfalfa Cultivars Under Climate-Induced Drought Stress

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- **Alfalfa is the most important forage crop in Romania. The main objectives in alfalfa breeding are: improving quality, dry matter yield, as well as biotic and abiotic stress tolerance.**
- **Abiotic stress conditions cause extensive losses to agricultural production worldwide especial in perennial crops, like is alfalfa.**
- **Under these conditions, nine new varieties of alfalfa were studied for production, quality and adaptability.**
- **In order to shorten the breeding process, in the last year also started works that include the selection assisted by molecular markers.**
- **The experiments were in the second year of vegetation, they were installed in the spring of 2024 and will be studied and in the next year.**

## Materials and methods

The new Romanian alfalfa cultivars are synthetic ones obtained by polycross method and derived from hybrids between Romanian germplasm and different foreign sources. They are constituted from different number of progenies, from 5 components (F 31005-1-23) to 22 components (F 31022-1-23 ). One of the most important aims of Romanian breeding programme in alfalfa was to utilized different sources of germoplasm in order to avoid genetic vulnerability, but in the same time we had in the attention that new cultivars must to have a good phenotypic uniformity in concordance with UPOV requirements. The trials were carried out in 2025, in 3 locations: NARDI Fundulea and ARDS Secuieni in dry land and ARDS Caracal under irrigated conditions.

The experimental design was a randomized complete blocks with 4 replications, with 15 $m^2$/plots in Fundulea and with 10 $m^2$/plots in Secuieni and Caracal.

## RESULTS

### The monthly rainfalls and temperatures recorded at NARDI Fundulea, ARDS Secuieni and ARDS Caracal in agricultural year 2024-2025

| Year | Month | | | | | | | | | | | | Sum/ average |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | X | XI | XII | I | II | III | IV | V | VI | VII | VIII | IX | |
| **Rainfall (mm)** | | | | | | | | | | | | | |
| **NARDI FUNDULEA** | | | | | | | | | | | | | |
| **2024-2025** | 7.8 | 49 | 85 | 10.8 | 22.8 | 25.6 | 29.6 | 89 | 9.2 | 42.6 | 4 | 10.8 | 386.2 |
| **Multi year average** | 42.3 | 42 | 44 | 35 | 32 | 37 | 45 | 63 | 75 | 71 | 50 | 49 | 584.3 |
| **Difference ±** | -34.5 | 7.0 | 41.0 | -24.2 | -9.2 | -11.4 | -15.4 | 26.0 | -65.8 | -28.4 | -46.0 | -38.2 | -198.1 |
| **ARDS SECUIENI** | | | | | | | | | | | | | |
| **2024-2025** | 35.8 | 21.4 | 36.2 | 12.2 | 4.2 | 32.6 | 12.4 | 116.4 | 8.5 | 73.2 | 8.6 | 31.2 | 392.7 |
| **Multi year average** | 36.9 | 27.7 | 25.4 | 19.6 | 19.2 | 26.3 | 44.9 | 64.3 | 84.7 | 80.6 | 58.0 | 44.3 | 531.9 |
| **Difference ±** | -1.1 | -6.3 | 10.8 | -7.4 | -15.0 | 6.3 | -32.5 | 52.1 | -76.2 | -7.4 | -49.4 | -13.1 | -139.2 |
| **ARDS CARACAL** | | | | | | | | | | | | | |
| **2024-2025** | 3.4 | 55.2 | 113.6 | 4.4 | 6.4 | 19 | 22 | 104.6 | 21.8 | 0 | 30.4 | 112 | 509.8 |
| **Multi year average** | 46 | 37 | 39.1 | 30.8 | 26.3 | 34.2 | 47.8 | 58.6 | 69.7 | 62.1 | 46.6 | 39.6 | 492.8 |
| **Difference ±** | -42.6 | 18.2 | 74.5 | -26.4 | -19.9 | -15.2 | -25.8 | 46 | -47.9 | -62.1 | -16.2 | 72.4 | +17.0 |
| **TEMPERATURE (°C)** | | | | | | | | | | | | | |
| **NARDI FUNDULEA** | | | | | | | | | | | | | |
| **2024-2025** | 16.1 | 8.5 | 4.3 | 1 | 7.6 | 8.5 | 15 | 16.4 | 26.1 | 27.7 | 26 | 20 | 14.79 |
| **Multi year average** | 11.3 | 5.4 | 0 | -2.4 | -0.4 | 4.9 | 11.3 | 17 | 20.8 | 22.7 | 22 | 17.5 | 10.87 |
| **Difference ±** | 4.8 | 3.1 | 4.3 | 3.4 | 7.2 | 3.6 | 3.7 | -0.6 | 5.3 | 5 | 4 | 2.5 | 3.92 |
| **ARDS SECUIENI** | | | | | | | | | | | | | |
| **2024-2025** | 10.2 | 2.8 | 1.4 | 1.9 | -2.5 | 7.4 | 10.5 | 12.9 | 20.6 | 22.2 | 20.9 | 16.8 | 10.4 |
| **Multi year average** | 9.2 | 3.6 | -1.5 | -3.7 | -1.9 | 2.8 | 9.6 | 15.4 | 18.9 | 20.4 | 19.7 | 15.1 | 9.0 |
| **Difference** | 1.0 | -0.8 | 2.9 | 5.6 | -0.6 | 4.6 | 0.9 | -2.5 | 1.7 | 1.8 | 1.2 | 1.7 | 1.5 |
| **ARDS CARACAL** | | | | | | | | | | | | | |
| **2024-2025** | 13.0 | 4.5 | 4.0 | 2.4 | -1.3 | 9.3 | 12.5 | 16.0 | 24.0 | 23.9 | 24.4 | 16.0 | +12.39 |
| **Multi year average** | 11.7 | 5.1 | 0.3 | -1.3 | 0.8 | 6.0 | 12.0 | 17.7 | 21.6 | 22.8 | 23.5 | 17.6 | +11.48 |
| **Difference** | 1.3 | -0.6 | 3.7 | 3.7 | -2.1 | 3.3 | 0.5 | -1.7 | 2.4 | 1.1 | 0.9 | -1.6 | +0.91 |

May 2025

August 2025

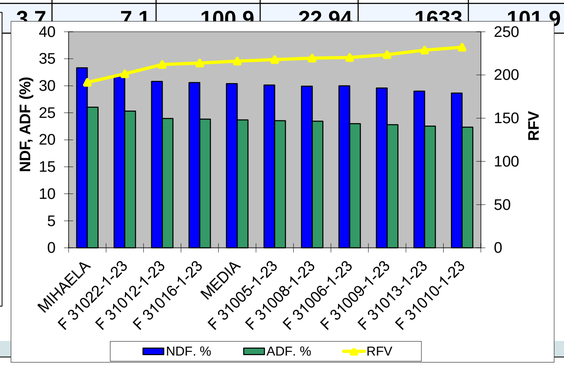
### Fodder yield achieved by new alfalfa cultivars developed in NARDI Fundulea, in 2025

| Genotype | NARDI Fundulea | ARDS Caracal | ARDS Secuieni | Average | |
|---|---|---|---|---|---|
| | (t $ha^{-1}$) | (t $ha^{-1}$) | (t $ha^{-1}$) | (t $ha^{-1}$) | % from control |
| **F 31009-1-2023** | **36.9** | **25.8** | **13.6** | **25.4** | **105.1** |
| **F 31005-1-2023** | **36.7** | **25.2** | **13.8** | **25.2** | **104.3** |
| **F 31016-1-2023** | **36.6** | **25.2** | **13.3** | **25.0** | **103.4** |
| **F 31006-1-2023** | **39.9** | **22.8** | **11.8** | **24.8** | **102.6** |
| **F 31013-1-2023** | **37.1** | **24.9** | **12.4** | **24.8** | **102.5** |
| **F 31010-1-2023** | **36.3** | **24.9** | **12.4** | **24.5** | **101.4** |
| **F 31008-1-2023** | **36.9** | **23.7** | **12.7** | **24.4** | **101.0** |
| **MIHAELA (Ct.)** | **36.5** | **24** | **12.1** | **24.2** | **100.0** |
| **F 31012-1-2023** | **36.5** | **23.4** | **12.5** | **24.1** | **99.7** |
| **F 31022-1-2023** | **37.4** | **24.6** | **10.36** | **24.1** | **99.7** |
| **Average** | **37.1** | **24.3** | **12.4** | **24.6** | **101.6** |
| **% from Fundulea** | **100.0** | **65.5** | **33.4** | **72.8** | |

### Dry matter and crude protein yield achieved by new alfalfa cultivars in the field test, in 2025

| Genotype | NARDI Fundulea | ARDS Caracal | ARDS Secuieni | Average | | C.P. from D.M. | Crude protein | |
|---|---|---|---|---|---|---|---|---|
| | (t $ha^{-1}$) | (t $ha^{-1}$) | (t $ha^{-1}$) | (t $ha^{-1}$) | % from ct. | % | (kg $ha^{-1}$) | % from ct. |
| **F 31009-1-2023** | **10.26** | **7.83** | **4.02** | **7.4** | **104.4** | **23.38** | **1723** | **107.5** |
| **F 31006-1-2023** | **11.08** | **7.11** | **3.62** | **7.3** | **103.0** | **23.67** | **1721** | **107.3** |
| **F 31013-1-2023** | **10.33** | **7.59** | **3.81** | **7.2** | **102.6** | **23.72** | **1718** | **107.2** |
| **F 31005-1-2023** | **10.21** | **7.77** | **4.07** | **7.4** | **104.2** | **22.70** | **1668** | **104.1** |
| **MIHAELA (Ct.)** | **10.15** | **7.23** | **3.58** | **7.0** | **99.0** | **22.95** | **1603** | **100.0** |
| **F 31012-1-2023** | **10.16** | **7.14** | **3.88** | **7.1** | **100.0** | **22.68** | **1601** | **99.9** |
| **F 31022-1-2023** | **10.41** | **7.53** | **3.09** | **7.0** | **99.3** | **22.72** | **1593** | **99.3** |
| **F 31010-1-2023** | **9.90** | **7.47** | **3.63** | **7.0** | **99.2** | **22.74** | **1592** | **99.3** |
| **F 31016-1-2023** | **10.17** | **7.35** | **3.83** | **7.1** | **100.9** | **22.35** | **1591** | **99.2** |
| **F 31008-1-2023** | **10.27** | **7.17** | **3.73** | **7.1** | **100.0** | **22.46** | **1585** | **98.8** |
| **Average** | | | **3.7** | **7.1** | **100.9** | **22.94** | **1633** | **101.9** |

Figure 1. The relationship between NDF, ADF and RFV




### Fodder quality of new synthetic alfalfa cultivars in 2025, developed in NARDI Fundulea

| Genotype | NDF. % | ADF. % | RFV | % Ch.) |
|---|---|---|---|---|
| **F 31010-1-2023** | **28.66** | **22.35** | **232.0** | **121.2** |
| **F 31013-1-2023** | **29.01** | **22.53** | **228.8** | **119.5** |
| **F 31009-1-2023** | **29.60** | **22.81** | **223.5** | **116.8** |
| **F 31006-1-2023** | **29.99** | **22.99** | **220.2** | **115.0** |
| **F 31008-1-2023** | **29.93** | **23.44** | **219.6** | **114.7** |
| **F 31005-1-2023** | **30.14** | **23.54** | **217.8** | **113.8** |
| **F 31016-1-2023** | **30.60** | **23.82** | **213.8** | **111.7** |
| **F 31012-1-2023** | **30.81** | **23.94** | **212.1** | **110.8** |
| **F 31022-1-2023** | **31.97** | **25.32** | **201.3** | **105.1** |
| **MIHAELA (Ct.)** | **33.34** | **26.04** | **191.4** | **100.0** |
| **MEDIA** | **30.41** | **23.68** | **216.1** | **112.9** |

### Seed yield of the new alfalfa cultivars in 2025

| Genotype | NARDI FUNDULEA | ARDS SECUIENI | AVERAGE | |
|---|---|---|---|---|
| | | | (kg $ha^{-1}$) | % from control |
| **F31013-1-2023** | **201** | **738** | **470** | **116** |
| **F31005-2-2023** | **256** | **698** | **477** | **118** |
| **F31008-1-2023** | **227** | **691** | **459** | **114** |
| **F31012-1-2023** | **211** | **683** | **447** | **111** |
| **F31010-1-2023** | **220** | **679** | **450** | **111** |
| **F31006-2-2023** | **229** | **667** | **448** | **111** |
| **F31016-1-2023** | **230** | **640** | **435** | **108** |
| **MIHAELA (Ct.)** | **223** | **584** | **404** | **100** |
| **F31009-1-2023** | **255** | **561** | **408** | **101** |
| **F31022-1-2023** | **232** | **553** | **393** | **97** |
| **Average** | **228** | **649** | **439** | **109** |

**In 2025, 20 alfalfa genotypes were analyzed. Molecular analyses consisted of continuing analyses with SSR markers and initiating analyses with KASP markers.**

**KASP markers were created, using the PolyMarker software, based on the sequences of some SNPs from the specialty literature (Medina et al., 2024), with localization within genes with a direct or indirect role in alfalfa defense against biotic and abiotic stress factors. In this phase, three KASP markers were analyzed: 3_74150027, 6_11528532 and 7_65295546.**

**Molecular analysis with marker 7_65295546 revealed the separation of the material into three groups (a, a2 and a3). The majority of genotypes being in group a2 (13 lines). Comparative analysis of molecular results with phenotypic ones will allow selection of favorable haplotypes.**

**Molecular analyses with 9 SSR markers (AW01, AW11, AW123, AW300, AW690665, BE112, M230, MTIC432 and MS27) revealed genetic variability in the analyzed alfalfa germplasm, observing markers that determined the visualization of multiple alleles at a single locus (BE112, AW690665, M230, MTIC432 and MS27) or with amplification of two loci (mtic432).**

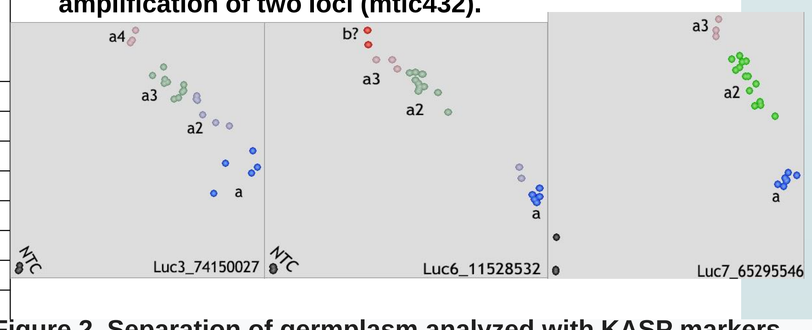



**Figure 2. Separation of germplasm analyzed with KASP markers.**

## CONCLUSIONS

- **The new Romanian alfalfa cultivars represent a genetic progress achieved in the alfalfa breeding program of NARDI Fundulea; all new cultivars demonstrated a good adaptability in the conditions of 2025, with long periods of drought and high temperatures. In this conditions, only two harvest was obtained for alfalfa in the second year of vegetation;**
- **The yields obtained in all three testing centers (Fundulea, Caracal, Secuieni) were very low and represented less than 50% of the potential of the alfalfa varieties.**
- **On average in the three test centers, the best behavior was the varieties: F 31009-1-24, F31006-1-24, F31013-1-24 and F 31005-1-24 with a production of 7.2-7.4 t $ha^{-1}$ dry matter and 1668- 1723 kg $ha^{-1}$ crude protein (4.1-7.5% more than Mihaela, the control variety;**
- **The new genotypes are characterized by very good quality (more than 20% C.P. and a relative food value of 201 - 236); respectively, SUPREME category feed, according to the US scale. The research aimed at the assisted selection of molecular markers for resistance/tolerance to diseases and drought, highlighted a great variability and creates the premises for their use in the alfalfa breeding program in Fundulea.**

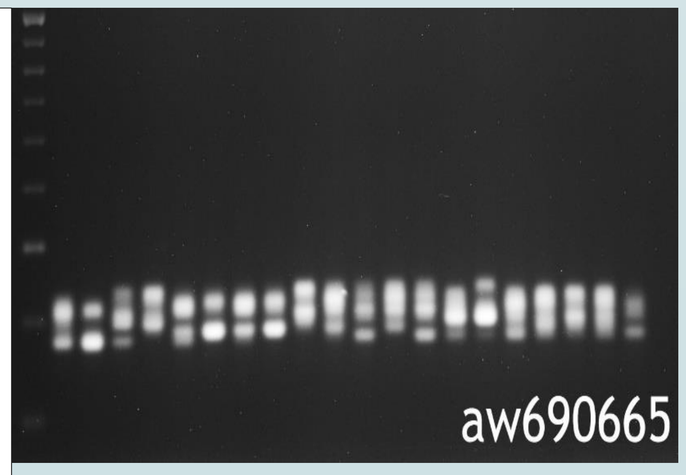



Figure 3. Molecular analyses with marker AW690665

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