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| Please use this form to clearly and concisely report on project progress. The information included should reflect quantifiable results that can be used to evaluate and measure project success. Comments should be limited to the designated boxes. Technical reports, no longer than 4 pages, may be attached to this summary report. |
| Project Number: |  |
| Project Title:  | Identification and confirmation of natural tolerance to off-target Dicamba damage in non-Xtend soybeans |
| Organization:  | University of Missouri |
| Principal Investigator Name: | Feng Lin |
| Other investigators: | Caio Viera |
| Report Period: | December 15, 2023, to March 15, 2024 |
| **Research plans for 2024**1) Selection of lines for release as soybean varieties or germplasm with excellent yield, dicamba tolerance and multiple disease resistance compared to current dicamba varieties2) Identify and evaluate MG3L to MG4L among 100 advanced and approximately 849 lines in preliminary yield tests for dicamba tolerance, favorable agronomic traits, and disease resistance.3) Continue development of high yielding populations for dicamba tolerance with high yield and disease resistance.4) Gene discovery for dicamba using the two mapping populations S16-9666 (susceptible) x PI 603497 tolerant) and S13-2743 (susceptible) x PI 567357 (tolerant)**OBJECTIVE 1:** Identification and selection of high-yielding tolerant advanced lines**1. Development of Enlist-E3 materials with off-target dicamba tolerance:** Seven populations are being developed at the University of Arkansas consisting of a high-yielding Enlist-E3 line and a non-GMO dicamba-tolerant source (Table 1). Crosses were made in off-season nurseries and are expected to be harvested by April 2024. Populations will be advanced until F4 and tested under off-target dicamba exposure in summer 2025. The goal is to develop MG 4 Enlist-E3 materials that can coexist in a predominantly dicamba-based cropping system.**Table 1**. Enlist-E3 materials being crossed to non-GMO dicamba-tolerant genetic sources.

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| **Name** | **Type** | **MG** | **2024 Trials** | **23 Yield** | **23 %E3 CK** |
| R23PR-00100 | Enlist-E3 | 4E | USDA UP 4E, AR-OVT | 61.9 | 101.5 |
| R23PR-00043 | Enlist-E3 | 4E | USDA UP 4E, AR-OVT | 68.2 | 107.0 |
| R23PR-00037 | Enlist-E3 | 4L | USDA UP 4L, AR-OVT | 69.2 | 106.6 |
| R23PR-00068 | Enlist-E3 | 4L | USDA UP 4L, AR-OVT | 65.2 | 107.0 |
| R23PR-00089 | Enlist-E3 | 4L | USDA UP 4L, AR-OVT | 67.6 | 93.2 |
| R23PR-00035 | Enlist-E3 | 5E | USDA UP 5E, AR-OVT | 68.5 | 107.5 |
| R23PR-00055 | Enlist-E3 | 5E | USDA UP 5E, AR-OVT | 70.1 | 107.9 |

**2. 2024 promising lines in regional trials:** We entered 17 high-yielding soybean lines of MG4E-MG4L genotype conferring different resistance and special traits such nematode, stem canker, frogeye leaf spot, cercospora leaf blight, drought, flood, high oleic, and Liberty Link traits in the 2024 USDA preliminary Southern yield trials (UP). Additionally, up to 8 promising breeding lines from MG4E-4L to MG5E, characterized by potential dicamba tolerance, were selected for the 2024 USDA Southern uniform yield trials (UT). These lines underwent prior exposure to dicamba in various trials conducted across the US Delta region and were specifically chosen based on their demonstrated high yield performance coupled with reduced damage and minimal yield loss attributable to dicamba exposure. The selected lines will be planted across two local environments at the Lee Farm Portageville, MO and in Fisk, MO (where dicamba incidence is minimal). These lines will be planted in the UP and UT as 3 replications across 9 to 15 diverse environments across different states including Tennessee, Arkansas, Mississippi, Alabama, Kansas, and Virginia.**3. 2024 advanced yield trials**: A total of 100 advanced breeding lines will be planted in Portageville, MO (2 local environments and 6-8 locations across different states (IL, AR, TN, MO). Among these lines, four derived from the cross between natural tolerant line S16-12774 with high yielding parents. **4. 2024 preliminary yield trials**: Around 849 soybean late III to Late IV breeding lines were visually selected based on pod load and agronomics. Those lines will be planted in Fisk, MO as 3 replications and in 2 other environments IL and AR.**5. 2024 progeny rows:** F4 single plant progenies from 7 crosses involving dicamba tolerant parents will be grown as a single row in the progeny testing nursery in 2024. **6. 2024 breeding populations advancement:** Sixteen conventional breeding populations to improve off target damage from Dicamba are being advanced from F1-F4 in winter nurseries in Costa Rica, which are expected to produce at least 1,600 new breeding lines to be evaluated in progeny rows in 2025. **7. 2024 crosses:** We aim to create 3-4 novel crosses by combining high-yielding lines with natural dicamba-tolerant line and plant introductions (PI) and in our breeding program.**OBJECTIVE 2**:Genomic studies to identify significant marker-trait associations to dicamba tolerance.**Dicamba QTL mapping populations:** Two RIL mapping populations (S16-9666 (susceptible) x PI 603497 tolerant) and S13-2743 (susceptible) x PI 567357 (tolerant)) were visually phenotyped at the R3-R4 growth stages for dicamba tolerance during the summer of 2023 in Arkansas and Missouri. During the summer of 2024, we will re-evaluate two RIL mapping populations for dicamba tolerance. Those RILs will be planted in AR and MO as single row with 3 replications. Combined data for two years (2023-2024) from tolerance visual ratings on each genotype will be used for detailed mapping analysis, providing a deeper comprehension of the genetic factors contributing to non-GMO tolerance.  |